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Preface

Welcome to *Microbiology*, an OpenStax resource. This textbook was written to increase student access to high-quality learning materials, maintaining highest standards of academic rigor at little to no cost.

About OpenStax

OpenStax is a nonprofit based at Rice University, and it's our mission to improve student access to education. Our first openly licensed college textbook was published in 2012, and our library has since scaled to over 20 books for college and AP[®] Courses used by hundreds of thousands of students. Our adaptive learning technology, designed to improve learning outcomes through personalized educational paths, is being piloted in college courses throughout the country. Through our partnerships with philanthropic foundations and our alliance with other educational resource organizations, OpenStax is breaking down the most common barriers to learning and empowering students and instructors to succeed.

About OpenStax Resources

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Format

You can access this textbook for free in web view or PDF through openstax.org, and for a low cost in print.

About *Microbiology*

Microbiology is designed to cover the scope and sequence requirements for the single-semester Microbiology course for non-majors. The book presents the core concepts of microbiology with a focus on applications for careers in allied health. The pedagogical features of *Microbiology* make the material interesting and accessible to students while maintaining the career-application focus and scientific rigor inherent in the subject matter.

Coverage and Scope

The scope and sequence of *Microbiology* has been developed and vetted with input from numerous instructors at institutions across the US. It is designed to meet the needs of most microbiology courses for non-majors and allied health students. In addition, we have also considered the needs of institutions that offer microbiology to a mixed audience of science majors and non-majors by frequently integrating topics that may not have obvious clinical relevance, such as environmental and applied microbiology and the history of science.

With these objectives in mind, the content of this textbook has been arranged in a logical progression from fundamental to more advanced concepts. The opening chapters present an overview of the discipline, with individual chapters focusing on microscopy and cellular biology as well as each of the classifications of microorganisms. Students then explore the foundations of microbial biochemistry, metabolism, and genetics, topics that provide a basis for understanding the various means by which we can control and combat microbial growth. Beginning with Chapter 15, the focus turns to microbial pathogenicity, emphasizing how interactions between microbes and the human immune system contribute to human health and disease. The last several chapters of the text provide a survey of medical microbiology, presenting the characteristics of microbial diseases organized by body system.

A brief Table of Contents follows. While we have made every effort to align the Table of Contents with the needs of our audience, we recognize that some instructors may prefer to teach topics in a different order. A particular strength of *Microbiology* is that instructors can customize the book, adapting it to the approach that works best in their classroom.

Chapter 1: An Invisible World

Chapter 2: How We See the Invisible World

Chapter 3: The Cell

Chapter 4: Prokaryotic Diversity

Chapter 5: The Eukaryotes of Microbiology

Chapter 6: Acellular Pathogens

Chapter 7: Microbial Biochemistry

Chapter 8: Microbial Metabolism

Chapter 9: Microbial Growth
Chapter 10: Biochemistry of the Genome
Chapter 11: Mechanisms of Microbial Genetics
Chapter 12: Modern Applications of Microbial Genetics
Chapter 13: Control of Microbial Growth
Chapter 14: Antimicrobial Drugs
Chapter 15: Microbial Mechanisms of Pathogenicity
Chapter 16: Disease and Epidemiology
Chapter 17: Innate Nonspecific Host Defenses
Chapter 18: Adaptive Specific Host Defenses
Chapter 19: Diseases of the Immune System
Chapter 20: Laboratory Analysis of the Immune Response
Chapter 21: Skin and Eye Infections
Chapter 22: Respiratory System Infections
Chapter 23: Urogenital System Infections
Chapter 24: Digestive System Infections
Chapter 25: Circulatory and Lymphatic System Infections
Chapter 26: Nervous System Infections
Appendix A: Fundamentals of Physics and Chemistry Important to Microbiology
Appendix B: Mathematical Basics
Appendix C: Metabolic Pathways
Appendix D: Taxonomy of Clinically Relevant Microorganisms
Appendix E: Glossary

American Society of Microbiology (ASM) Partnership

Microbiology is produced through a collaborative publishing agreement between OpenStax and the American Society for Microbiology Press. The book has been developed to align to the curriculum guidelines of the American Society for Microbiology.

About ASM

The American Society for Microbiology is the largest single life science society, composed of over 47,000 scientists and health professionals. ASM's mission is to promote and advance the microbial sciences.

ASM advances the microbial sciences through conferences, publications, certifications, and educational opportunities. It enhances laboratory capacity around the globe through training and resources and provides a network for scientists in academia, industry, and clinical settings. Additionally, ASM promotes a deeper understanding of the microbial sciences to diverse audiences and is committed to offering open-access materials through their new journals, American Academy of Microbiology reports, and textbooks.

ASM Recommended Curriculum Guidelines for Undergraduate Microbiology Education

PART 1: Concepts and Statements

Evolution

1. Cells, organelles (e.g., mitochondria and chloroplasts) and all major metabolic pathways evolved from early prokaryotic cells.
2. Mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms.
3. Human impact on the environment influences the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).
4. The traditional concept of species is not readily applicable to microbes due to asexual reproduction and the frequent occurrence of horizontal gene transfer.
5. The evolutionary relatedness of organisms is best reflected in phylogenetic trees.

Cell Structure and Function

6. The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).

7. Bacteria have unique cell structures that can be targets for antibiotics, immunity and phage infection.
8. Bacteria and Archaea have specialized structures (e.g., flagella, endospores, and pili) that often confer critical capabilities.
9. While microscopic eukaryotes (for example, fungi, protozoa and algae) carry out some of the same processes as bacteria, many of the cellular properties are fundamentally different.
10. The replication cycles of viruses (lytic and lysogenic) differ among viruses and are determined by their unique structures and genomes.

Metabolic Pathways

11. Bacteria and Archaea exhibit extensive, and often unique, metabolic diversity (e.g., nitrogen fixation, methane production, anoxygenic photosynthesis).
12. The interactions of microorganisms among themselves and with their environment are determined by their metabolic abilities (e.g., quorum sensing, oxygen consumption, nitrogen transformations).
13. The survival and growth of any microorganism in a given environment depends on its metabolic characteristics.
14. The growth of microorganisms can be controlled by physical, chemical, mechanical, or biological means.

Information Flow and Genetics

15. Genetic variations can impact microbial functions (e.g., in biofilm formation, pathogenicity and drug resistance).
16. Although the central dogma is universal in all cells, the processes of replication, transcription, and translation differ in Bacteria, Archaea, and Eukaryotes.
17. The regulation of gene expression is influenced by external and internal molecular cues and/or signals.
18. The synthesis of viral genetic material and proteins is dependent on host cells.
19. Cell genomes can be manipulated to alter cell function.

Microbial Systems

20. Microorganisms are ubiquitous and live in diverse and dynamic ecosystems.
21. Most bacteria in nature live in biofilm communities.
22. Microorganisms and their environment interact with and modify each other.
23. Microorganisms, cellular and viral, can interact with both human and nonhuman hosts in beneficial, neutral or detrimental ways.

Impact of Microorganisms

24. Microbes are essential for life as we know it and the processes that support life (e.g., in biogeochemical cycles and plant and/or animal microbiota).
25. Microorganisms provide essential models that give us fundamental knowledge about life processes.
26. Humans utilize and harness microorganisms and their products.
27. Because the true diversity of microbial life is largely unknown, its effects and potential benefits have not been fully explored.

PART 2: Competencies and Skills

Scientific Thinking

28. Ability to apply the process of science
 - a. Demonstrate an ability to formulate hypotheses and design experiments based on the scientific method.
 - b. Analyze and interpret results from a variety of microbiological methods and apply these methods to analogous situations.
29. Ability to use quantitative reasoning
 - a. Use mathematical reasoning and graphing skills to solve problems in microbiology.
30. Ability to communicate and collaborate with other disciplines
 - a. Effectively communicate fundamental concepts of microbiology in written and oral format.
 - b. Identify credible scientific sources and interpret and evaluate the information therein.
31. Ability to understand the relationship between science and society
 - a. Identify and discuss ethical issues in microbiology.

Microbiology Laboratory Skills

32. Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).
33. Use pure culture and selective techniques to enrich for and isolate microorganisms.
34. Use appropriate methods to identify microorganisms (media-based, molecular and serological).
35. Estimate the number of microorganisms in a sample (using, for example, direct count, viable plate count, and spectrophotometric methods).
36. Use appropriate microbiological and molecular lab equipment and methods.
37. Practice safe microbiology, using appropriate protective and emergency procedures.
38. Document and report on experimental protocols, results and conclusions.

OpenStax *Microbiology* Correlation to ASM Recommended Curriculum Guidelines for Undergraduate Microbiology Education

OpenStax <i>Microbiology</i> Correlation to ASM Curriculum Guidelines	
Chapter	ASM Curriculum Guidelines
1—An Invisible World	2, 4, 5, 11, 16, 20, 23, 26, 27, 31
2—How We See the Invisible World	6, 31, 32, 33

OpenStax *Microbiology* Correlation to ASM Curriculum Guidelines

Chapter	ASM Curriculum Guidelines
3—The Cell	1, 2, 5, 9, 16, 21, 25, 31
4—Prokaryotic Diversity	2, 4, 8, 11, 12, 16, 20, 23, 24, 31
5—The Eukaryotes of Microbiology	2, 4, 5, 9, 12, 20, 23, 31
6—Acellular Pathogens	4, 10, 18, 23, 31
7—Microbial Biochemistry	1, 24, 33, 34
8—Microbial Metabolism	1, 11, 12, 13, 22, 24
9—Microbial Growth	12, 13, 29, 31, 33, 34, 35
10—Biochemistry of the Genome	1, 16, 25, 31
11—Mechanisms of Microbial Genetics	1, 2, 15, 16, 17, 31
12—Modern Applications of Microbial Genetics	19, 26, 31
13—Control of Microbial Growth	13, 14, 26, 31, 36, 37
14—Antimicrobial Drugs	3, 7, 14, 15, 23, 26, 31
15—Microbial Mechanisms of Pathogenicity	8, 9, 10, 15, 18, 23, 33

OpenStax <i>Microbiology</i> Correlation to ASM Curriculum Guidelines	
Chapter	ASM Curriculum Guidelines
16—Disease and Epidemiology	7, 14, 23, 26, 31
17—Innate Nonspecific Host Defenses	7, 8, 23
18—Adaptive Specific Host Defenses	7, 23, 26, 31
19—Diseases of the Immune System	7, 8, 24
20—Laboratory Analysis of the Immune Response	31, 34
21—Skin and Eye Infections	8, 9, 10, 14, 18, 23, 24, 31
22—Respiratory System Infections	7, 8, 9, 14, 18, 23, 24, 31
23—Urogenital System Infections	7, 8, 9, 12, 14, 18, 22, 23, 24, 31
24—Digestive System Infections	7, 8, 9, 10, 14, 18, 23, 24, 31
25—Circulatory and Lymphatic System Infections	7, 8, 9, 14, 23, 31
26—Nervous System Infections	7, 8, 9, 14, 18, 23, 24, 31

Engaging Feature Boxes

Throughout *Microbiology*, you will find features that engage students by taking selected topics a step further. Our features include:

Clinical Focus. Each chapter has a multi-part clinical case study that follows the story of a fictional patient. The case unfolds in several realistic episodes placed strategically throughout the chapter, each episode revealing new symptoms and clues about possible causes and diagnoses. The details of the case are directly related to the topics presented in the chapter, encouraging students to apply what they are learning to real-life scenarios. The final episode presents a Resolution that reveals the outcome of the case and unpacks the broader lessons to be learned.

Case in Point. In addition to the Clinical Focus, many chapters also have one or more single-part case studies that serve to highlight the clinical relevance of a particular topic. These narratives are strategically placed directly after the topic of emphasis and generally conclude with a set of questions that challenge the reader to think critically about the case.

Micro Connections. All chapters contain several Micro Connections feature boxes that highlight real-world applications of microbiology, drawing often-overlooked connections between microbiology and a wide range of other disciplines. While many of these connections involve medicine and healthcare, they also venture into domains such as environmental science, genetic engineering, and emerging technologies. Moreover, many Micro Connections boxes are related to current or recent events, further emphasizing the intersections between microbiology and everyday life.

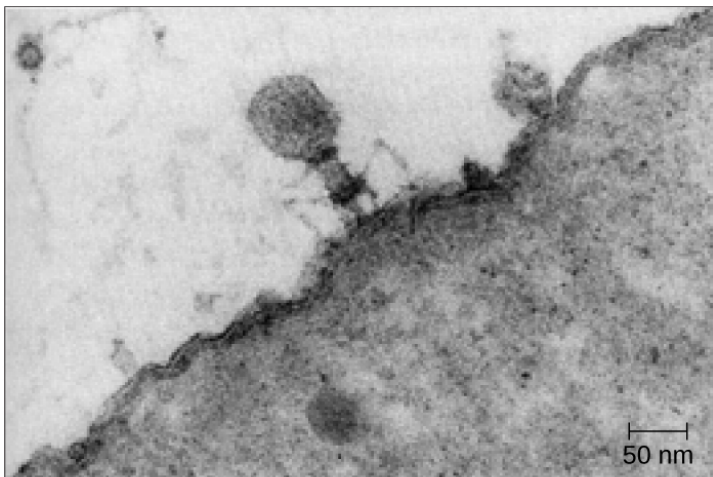
Sigma Xi Eye on Ethics. This unique feature, which appears in most chapters, explores an ethical issue related to chapter content. Developed in cooperation with the scientific research society Sigma Xi, each Eye on Ethics box presents students with a challenging ethical dilemma that arises at the intersection of science and healthcare. Often grounded in historical or current events, these short essays discuss multiple sides of an issue, posing questions that challenge the reader to contemplate the ethical principles that govern professionals in healthcare and the sciences.

Disease Profile. This feature, which is exclusive to Chapters 21–26, highlights important connections between related diseases. Each box also includes a table cataloguing unique aspects of each disease, such as the causative agent, symptoms, portal of entry, mode of transmission, and treatment. These concise tables serve as a useful reference that students can use as a study aid.

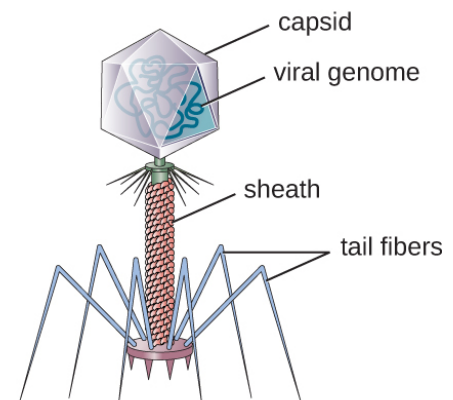
Link to Learning. This feature provides a brief introduction and a link to an online resource that students may use to further explore a topic presented in the chapter. Links typically lead to a website, interactive activity, or animation that students can investigate on their own.

Comprehensive Art Program

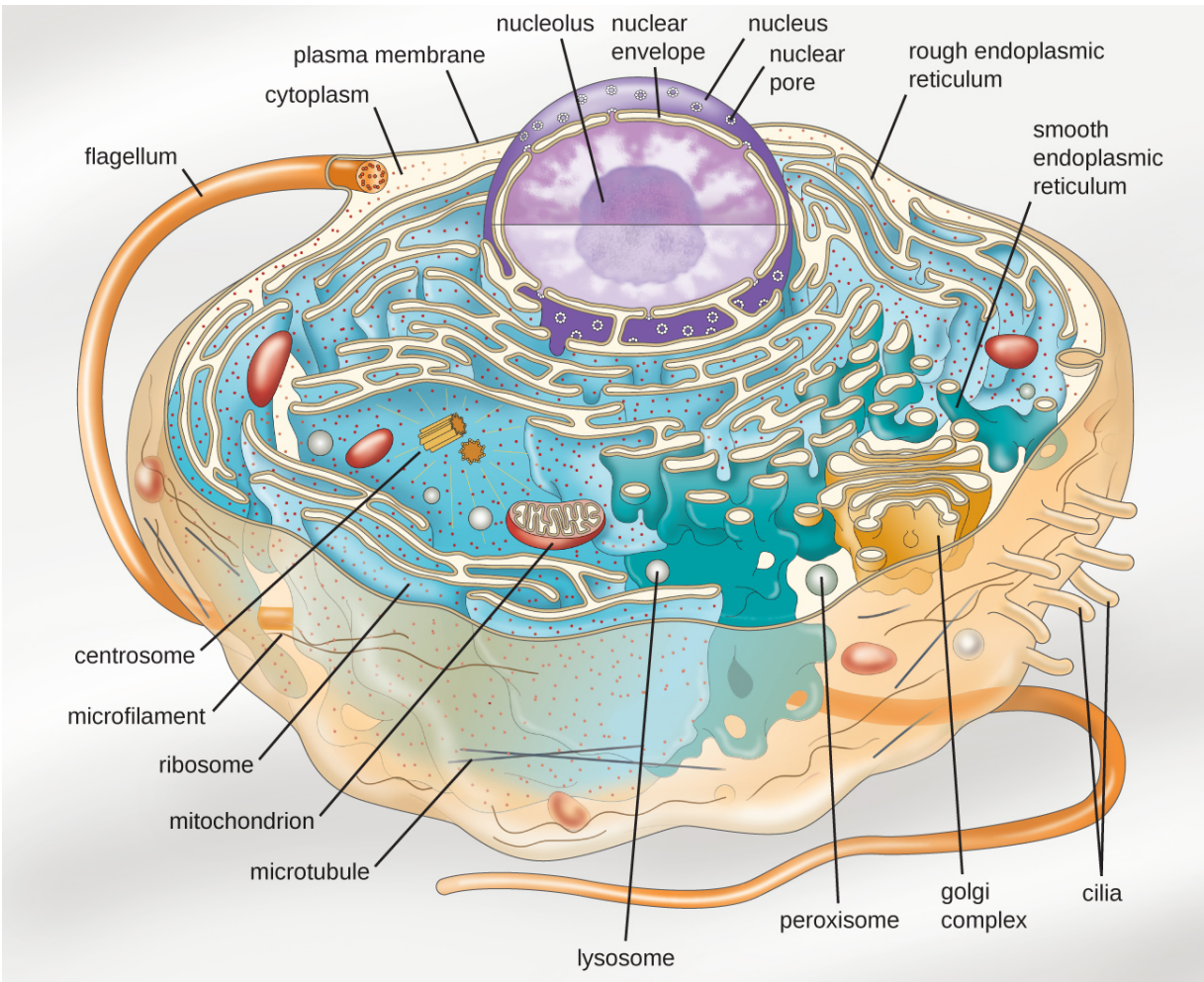
Our art program is designed to enhance students' understanding of concepts through clear and effective illustrations, diagrams, and photographs. Detailed drawings, comprehensive lifecycles, and clear micrographs provide visual reinforcement for concepts.



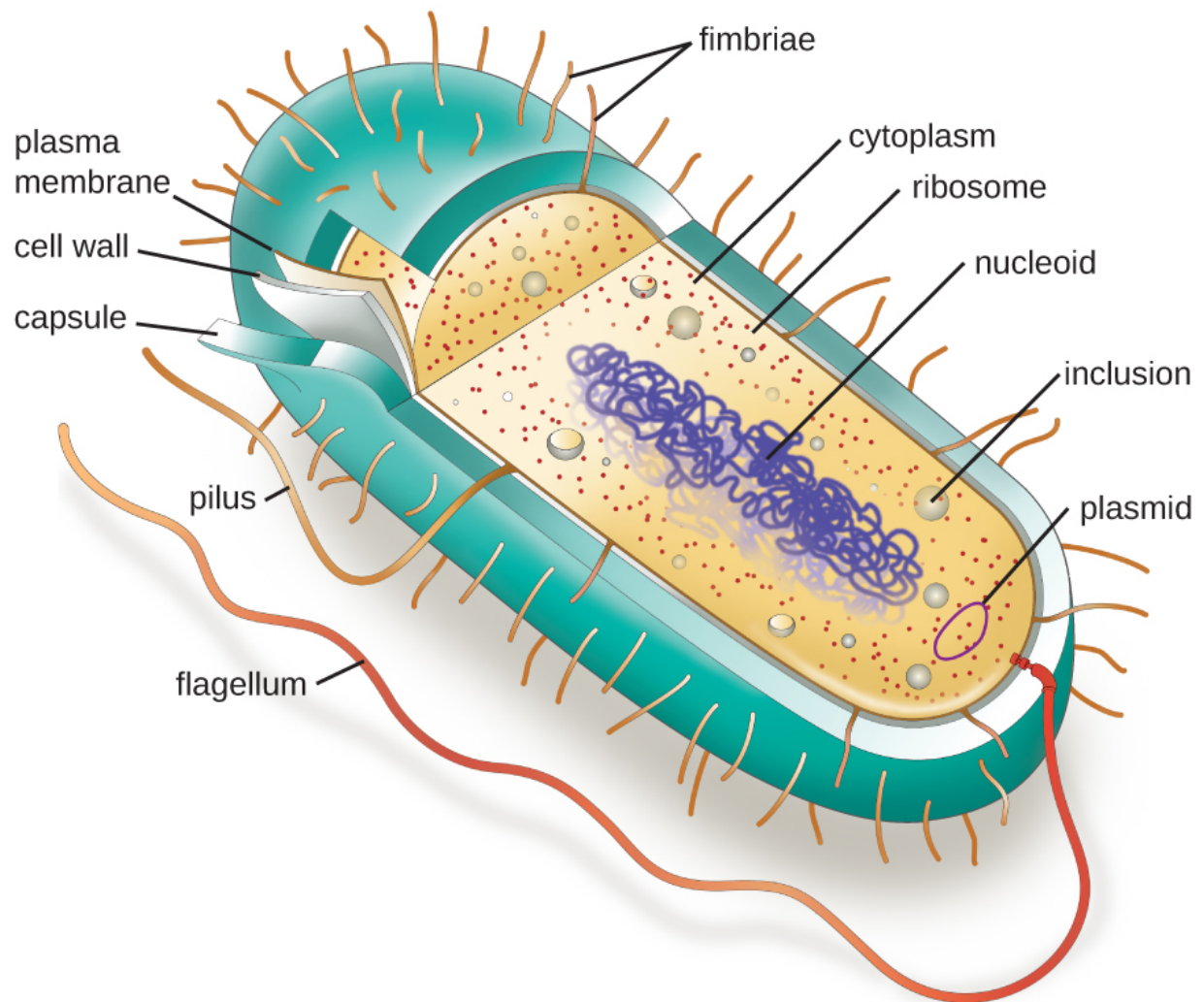
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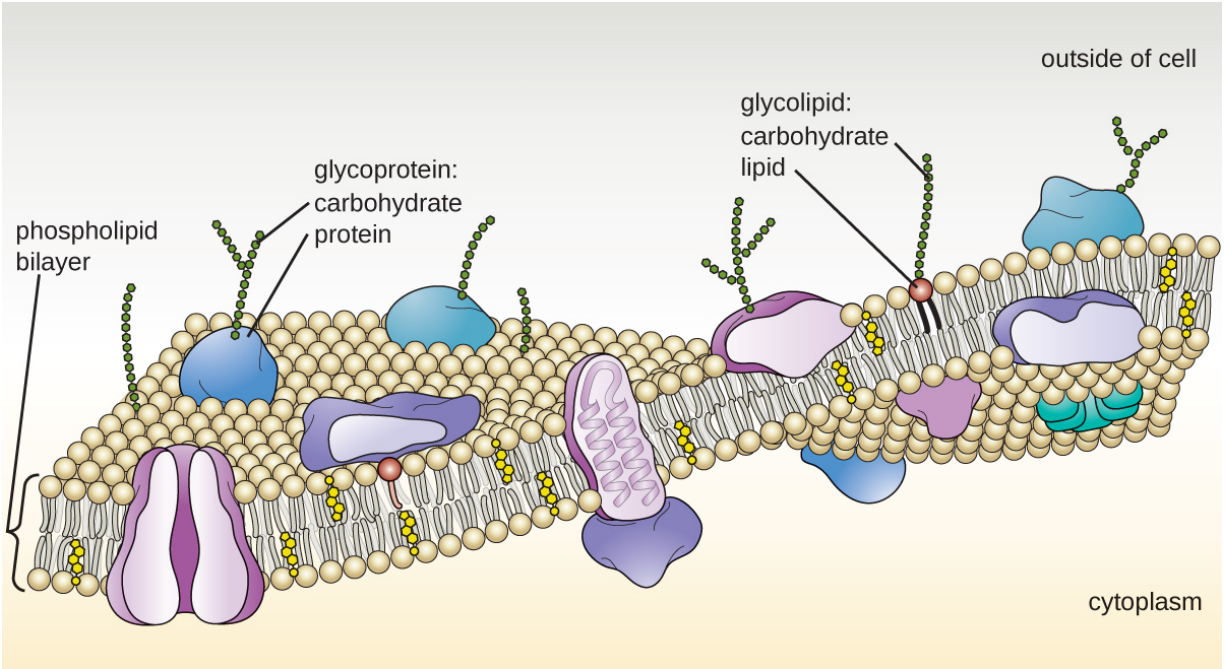


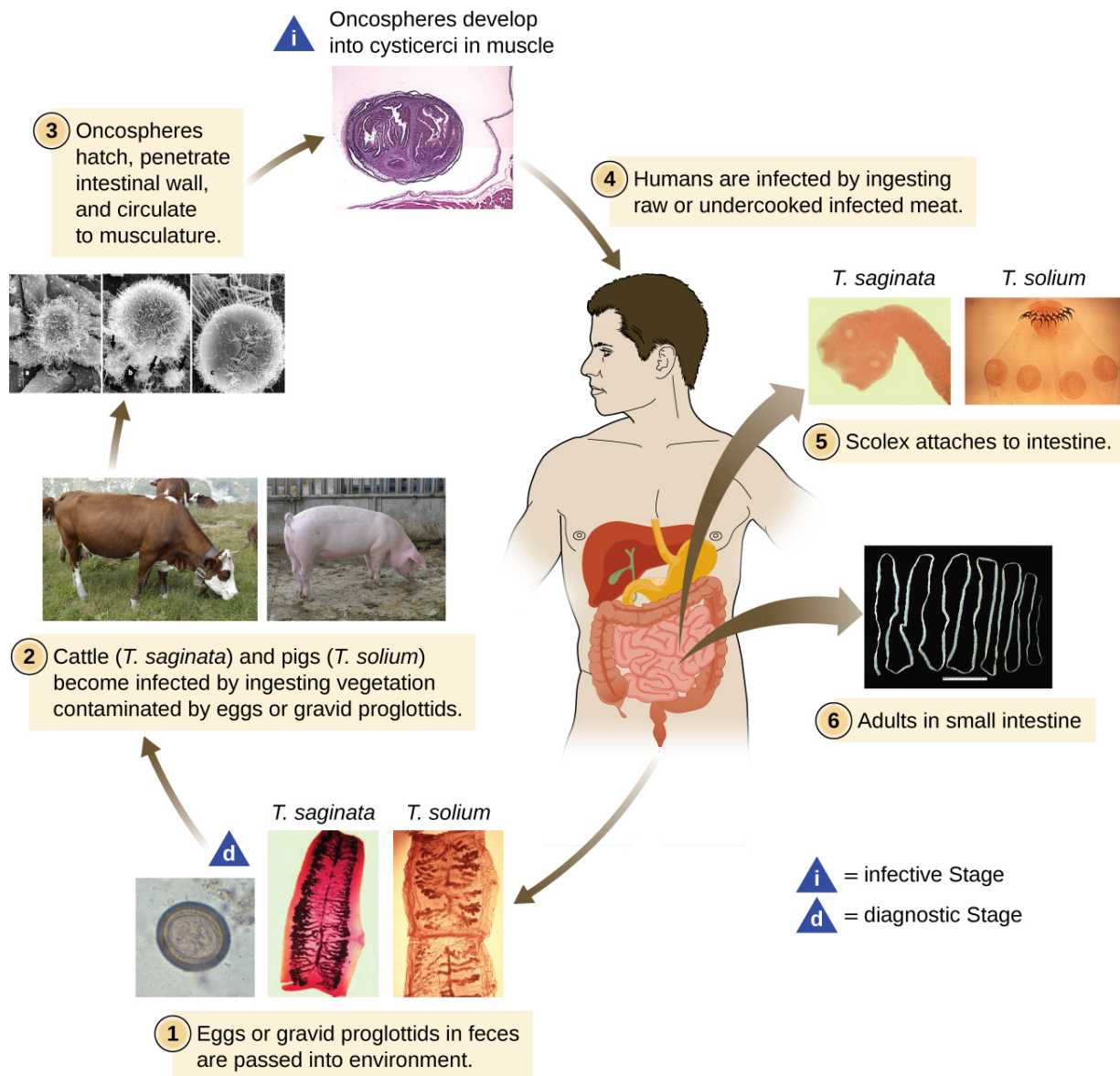
(b)



ELECTRON MICROSCOPES Magnification: 20–100,000× or more		
Use electron beams focused with magnets to produce an image.		
Microscope Type	Key Uses	Sample Images
Transmission (TEM)	Uses electron beams that pass through a specimen to visualize small images; useful to observe small, thin specimens such as tissue sections and subcellular structures. Example: <i>Ebola virus</i>	
Scanning (SEM)	Uses electron beams to visualize surfaces; useful to observe the three-dimensional surface details of specimens. Example: <i>Campylobacter jejuni</i>	







Materials That Reinforce Key Concepts

Learning Objectives. Every section begins with a set of clear and concise learning objectives that are closely aligned to the content and Review Questions.

Summary. The Summary distills the information in each section into a series of concise bullet points. Key Terms in the Summary are bold-faced for emphasis.

Key Terms. New vocabulary is bold-faced when first introduced in the text and followed by a definition in context. Definitions of key terms are also listed in the Glossary in ([Appendix E](#)).

Check Your Understanding questions. Each subsection of the text is punctuated by one or more comprehension-level questions. These questions encourage readers to make sure they understand what they have read before moving on to the next topic.

Review Questions. Each chapter has a robust set of review questions that assesses students' mastery of the Learning Objectives. Questions are organized by format: multiple choice, matching, true/false, fill-in-the-blank, short answer, and critical thinking.

Additional Resources

Student and Instructor Resources

We've compiled additional resources for both students and instructors, including Getting Started Guides, a test bank, and an instructor answer guide. Instructor resources require a verified instructor account, which can be requested on your openstax.org log-in. Take advantage of these resources to supplement your OpenStax book.

Partner Resources

OpenStax Partners are our allies in the mission to make high-quality learning materials affordable and accessible to students and instructors everywhere. Their tools integrate seamlessly with our OpenStax titles at a low cost. To access the partner resources for your text, visit your book page on openstax.org.

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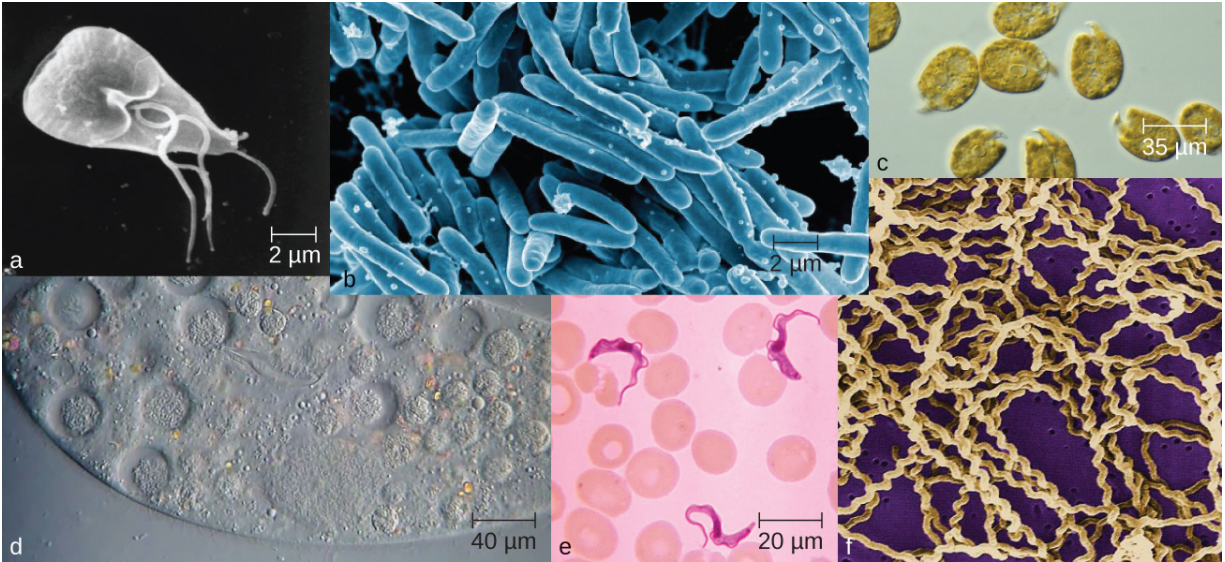
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Introduction

class="introduction"

Microorganisms vary visually in their size and shape, as can be observed microscopically; but they also vary in invisible ways, such as in their metabolic capabilities.

(credit a, e, f: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by NIAID; credit c: modification of work by CSIRO; credit d: modification of work by “Microscopic World”/YouTube)



Life takes many forms, from giant redwood trees towering hundreds of feet in the air to the tiniest known microbes, which measure only a few billionths of a meter. Humans have long pondered life's origins and debated the defining characteristics of life, but our understanding of these concepts has changed radically since the invention of the microscope. In the 17th century, observations of microscopic life led to the development of the cell theory: the idea that the fundamental unit of life is the cell, that all organisms contain at least one cell, and that cells only come from other cells.

Despite sharing certain characteristics, cells may vary significantly. The two main types of cells are prokaryotic cells (lacking a nucleus) and eukaryotic cells (containing a well-organized, membrane-bound nucleus). Each type of cell exhibits remarkable variety in structure, function, and metabolic activity ([\[link\]](#)). This chapter will focus on the historical discoveries that have shaped our current understanding of microbes, including their origins and their role in human disease. We will then explore the distinguishing structures found in prokaryotic and eukaryotic cells.

Spontaneous Generation

LEARNING OBJECTIVES

- Explain the theory of spontaneous generation and why people once accepted it as an explanation for the existence of certain types of organisms
- Explain how certain individuals (van Helmont, Redi, Needham, Spallanzani, and Pasteur) tried to prove or disprove spontaneous generation

Note:

Part 1

Barbara is a 19-year-old college student living in the dormitory. In January, she came down with a sore throat, headache, mild fever, chills, and a violent but unproductive (i.e., no mucus) cough. To treat these symptoms, Barbara began taking an over-the-counter cold medication, which did not seem to work. In fact, over the next few days, while some of Barbara's symptoms began to resolve, her cough and fever persisted, and she felt very tired and weak.

- What types of respiratory disease may be responsible?

Jump to the [next](#) Clinical Focus box

Humans have been asking for millennia: Where does new life come from? Religion, philosophy, and science have all wrestled with this question. One

of the oldest explanations was the theory of spontaneous generation, which can be traced back to the ancient Greeks and was widely accepted through the Middle Ages.

The Theory of Spontaneous Generation

The Greek philosopher Aristotle (384–322 BC) was one of the earliest recorded scholars to articulate the theory of **spontaneous generation**, the notion that life can arise from nonliving matter. Aristotle proposed that life arose from nonliving material if the material contained *pneuma* (“vital heat”). As evidence, he noted several instances of the appearance of animals from environments previously devoid of such animals, such as the seemingly sudden appearance of fish in a new puddle of water.[\[footnote\]](#)

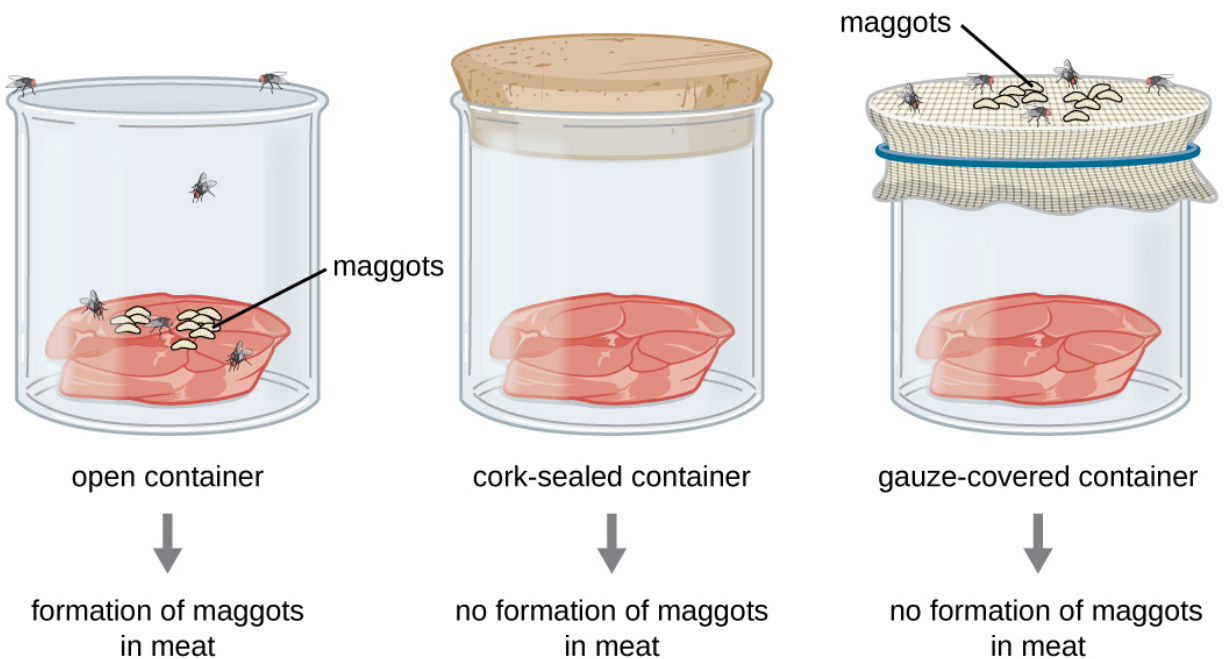
K. Zwier. “Aristotle on Spontaneous Generation.”

<http://www.sju.edu/int/academics/cas/resources/gppc/pdf/Karen%20R.%20Zwier.pdf>

This theory persisted into the 17th century, when scientists undertook additional experimentation to support or disprove it. By this time, the proponents of the theory cited how frogs simply seem to appear along the muddy banks of the Nile River in Egypt during the annual flooding. Others observed that mice simply appeared among grain stored in barns with thatched roofs. When the roof leaked and the grain molded, mice appeared. Jan Baptista van Helmont, a 17th century Flemish scientist, proposed that mice could arise from rags and wheat kernels left in an open container for 3 weeks. In reality, such habitats provided ideal food sources and shelter for mouse populations to flourish.

However, one of van Helmont’s contemporaries, Italian physician Francesco Redi (1626–1697), performed an experiment in 1668 that was one of the first to refute the idea that maggots (the larvae of flies) spontaneously generate on meat left out in the open air. He predicted that preventing flies from having direct contact with the meat would also prevent the appearance of maggots. Redi left meat in each of six containers ([\[link\]](#)). Two were open to the air, two were covered with gauze, and two were tightly sealed. His hypothesis was supported when maggots developed in the uncovered jars, but no maggots appeared in either the gauze-covered

or the tightly sealed jars. He concluded that maggots could only form when flies were allowed to lay eggs in the meat, and that the maggots were the offspring of flies, not the product of spontaneous generation.



Francesco Redi's experimental setup consisted of an open container, a container sealed with a cork top, and a container covered in mesh that let in air but not flies. Maggots only appeared on the meat in the open container. However, maggots were also found on the gauze of the gauze-covered container.

In 1745, John Needham (1713–1781) published a report of his own experiments, in which he briefly boiled broth infused with plant or animal matter, hoping to kill all preexisting microbes. [\[footnote\]](#) He then sealed the flasks. After a few days, Needham observed that the broth had become cloudy and a single drop contained numerous microscopic creatures. He argued that the new microbes must have arisen spontaneously. In reality, however, he likely did not boil the broth enough to kill all preexisting microbes.

E. Capanna. “Lazzaro Spallanzani: At the Roots of Modern Biology.” *Journal of Experimental Zoology* 285 no. 3 (1999):178–196.

Lazzaro Spallanzani (1729–1799) did not agree with Needham’s conclusions, however, and performed hundreds of carefully executed experiments using heated broth.[\[footnote\]](#) As in Needham’s experiment, broth in sealed jars and unsealed jars was infused with plant and animal matter. Spallanzani’s results contradicted the findings of Needham: Heated but sealed flasks remained clear, without any signs of spontaneous growth, unless the flasks were subsequently opened to the air. This suggested that microbes were introduced into these flasks from the air. In response to Spallanzani’s findings, Needham argued that life originates from a “life force” that was destroyed during Spallanzani’s extended boiling. Any subsequent sealing of the flasks then prevented new life force from entering and causing spontaneous generation ([\[link\]](#)).

R. Mancini, M. Nigro, G. Ippolito. “Lazzaro Spallanzani and His Refutation of the Theory of Spontaneous Generation.” *Le Infezioni in Medicina* 15 no. 3 (2007):199–206.



(a)



(b)



(c)

(a) Francesco Redi, who demonstrated that maggots were the offspring of flies, not products of spontaneous generation. (b) John Needham, who argued that microbes arose spontaneously in broth from a “life force.” (c) Lazzaro Spallanzani, whose experiments with broth aimed to disprove those of Needham.

Note:

- Describe the theory of spontaneous generation and some of the arguments used to support it.
- Explain how the experiments of Redi and Spallanzani challenged the theory of spontaneous generation.

Disproving Spontaneous Generation

The debate over spontaneous generation continued well into the 19th century, with scientists serving as proponents of both sides. To settle the debate, the Paris Academy of Sciences offered a prize for resolution of the problem. Louis Pasteur, a prominent French chemist who had been studying microbial fermentation and the causes of wine spoilage, accepted the challenge. In 1858, Pasteur filtered air through a gun-cotton filter and, upon microscopic examination of the cotton, found it full of microorganisms, suggesting that the exposure of a broth to air was not introducing a “life force” to the broth but rather airborne microorganisms.

Later, Pasteur made a series of flasks with long, twisted necks (“swan-neck” flasks), in which he boiled broth to sterilize it ([\[link\]](#)). His design allowed air inside the flasks to be exchanged with air from the outside, but prevented the introduction of any airborne microorganisms, which would get caught in the twists and bends of the flasks’ necks. If a life force besides the airborne microorganisms were responsible for microbial growth within the sterilized flasks, it would have access to the broth, whereas the microorganisms would not. He correctly predicted that sterilized broth in his swan-neck flasks would remain sterile as long as the swan necks remained intact. However, should the necks be broken, microorganisms

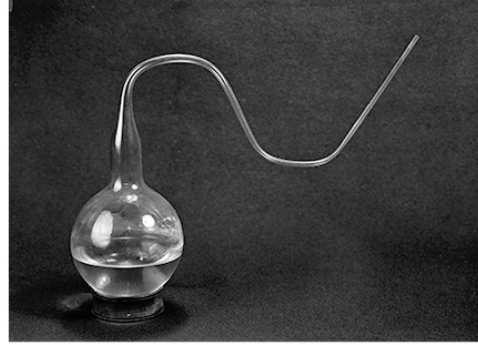
would be introduced, contaminating the flasks and allowing microbial growth within the broth.

Pasteur's set of experiments irrefutably disproved the theory of spontaneous generation and earned him the prestigious Alhumbert Prize from the Paris Academy of Sciences in 1862. In a subsequent lecture in 1864, Pasteur articulated "*Omne vivum ex vivo*" ("Life only comes from life"). In this lecture, Pasteur recounted his famous swan-neck flask experiment, stating that "...life is a germ and a germ is life. Never will the doctrine of spontaneous generation recover from the mortal blow of this simple experiment." [\[footnote\]](#) To Pasteur's credit, it never has.

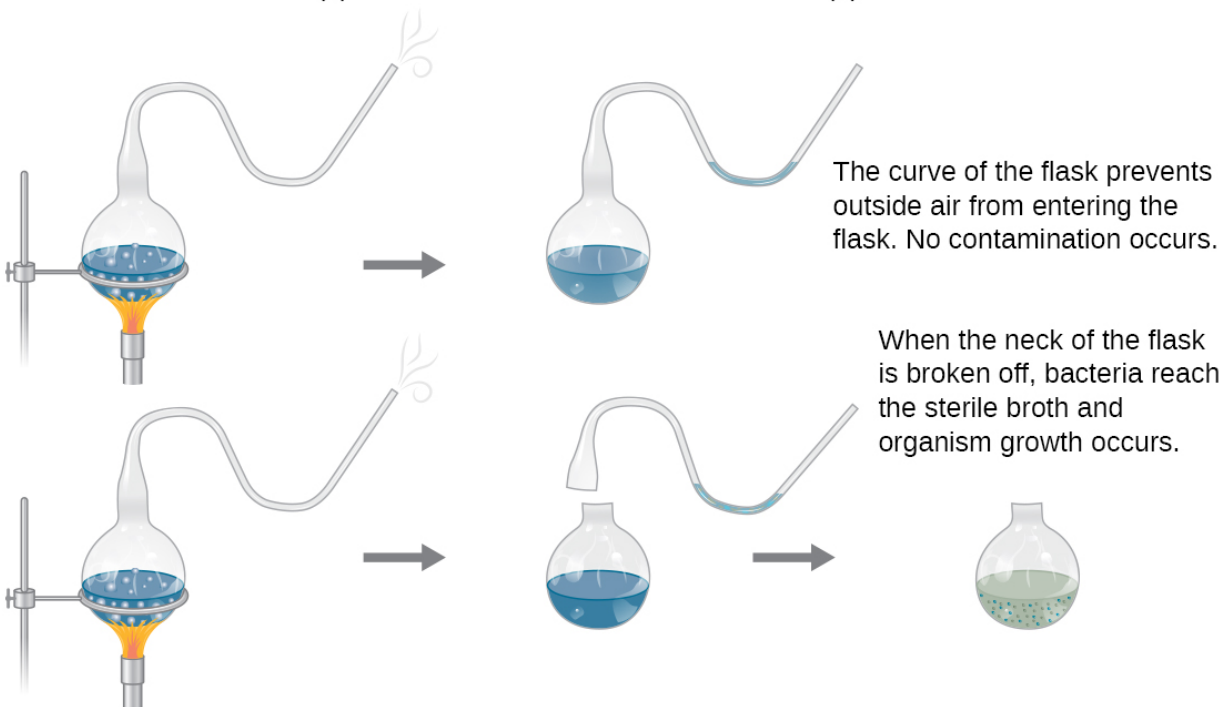
R. Vallery-Radot. *The Life of Pasteur*, trans. R.L. Devonshire. New York: McClure, Phillips and Co, 1902, 1:142.



(a)



(b)



Boiling the broth kills microorganisms.

The curve of the flask prevents outside air from entering the flask. No contamination occurs.

When the neck of the flask is broken off, bacteria reach the sterile broth and organism growth occurs.

(c)

(a) French scientist Louis Pasteur, who definitively refuted the long-disputed theory of spontaneous generation. (b) The unique swan-neck feature of the flasks used in Pasteur's experiment allowed air to enter the flask but prevented the entry of bacterial and fungal spores. (c) Pasteur's experiment consisted of two parts. In the first part, the broth in the flask was boiled to sterilize it. When this broth was cooled, it remained free of contamination. In the second part of the experiment, the flask was boiled and then the neck was broken off. The broth in

this flask became contaminated. (credit b: modification of work by “Wellcome Images”/Wikimedia Commons)

Note:

- How did Pasteur’s experimental design allow air, but not microbes, to enter, and why was this important?
- What was the control group in Pasteur’s experiment and what did it show?

Key Concepts and Summary

- The theory of **spontaneous generation** states that life arose from nonliving matter. It was a long-held belief dating back to Aristotle and the ancient Greeks.
- Experimentation by Francesco Redi in the 17th century presented the first significant evidence refuting spontaneous generation by showing that flies must have access to meat for maggots to develop on the meat. Prominent scientists designed experiments and argued both in support of (John Needham) and against (Lazzaro Spallanzani) spontaneous generation.
- Louis Pasteur is credited with conclusively disproving the theory of spontaneous generation with his famous swan-neck flask experiment. He subsequently proposed that “life only comes from life.”

Multiple Choice

Exercise:

Problem:

Which of the following individuals argued in favor of the theory of spontaneous generation?

- A. Francesco Redi
- B. Louis Pasteur
- C. John Needham
- D. Lazzaro Spallanzani

Solution:

C

Exercise:**Problem:**

Which of the following individuals is credited for definitively refuting the theory of spontaneous generation using broth in swan-neck flask?

- A. Aristotle
- B. Jan Baptista van Helmont
- C. John Needham
- D. Louis Pasteur

Solution:

D

Exercise:**Problem:**

Which of the following scientists experimented with raw meat, maggots, and flies in an attempt to disprove the theory of spontaneous generation?

- A. Aristotle
- B. Lazzaro Spallanzani
- C. Antonie van Leeuwenhoek
- D. Francesco Redi

Solution:

D

Fill in the Blank

Exercise:

Problem:

The assertion that “life only comes from life” was stated by Louis Pasteur in regard to his experiments that definitively refuted the theory of _____.

Solution:

spontaneous generation

True/False

Exercise:

Problem: Exposure to air is necessary for microbial growth.

Solution:

False

Short Answer

Exercise:**Problem:**

Explain in your own words Pasteur's swan-neck flask experiment.

Exercise:**Problem:**

Explain why the experiments of Needham and Spallanzani yielded in different results even though they used similar methodologies.

Critical Thinking**Exercise:****Problem:**

What would the results of Pasteur's swan-neck flask experiment have looked like if they supported the theory of spontaneous generation?

Foundations of Modern Cell Theory

LEARNING OBJECTIVES

- Explain the key points of cell theory and the individual contributions of Hooke, Schleiden, Schwann, Remak, and Virchow
- Explain the key points of endosymbiotic theory and cite the evidence that supports this concept
- Explain the contributions of Semmelweis, Snow, Pasteur, Lister, and Koch to the development of germ theory

While some scientists were arguing over the theory of spontaneous generation, other scientists were making discoveries leading to a better understanding of what we now call the cell theory. Modern cell theory has two basic tenets:

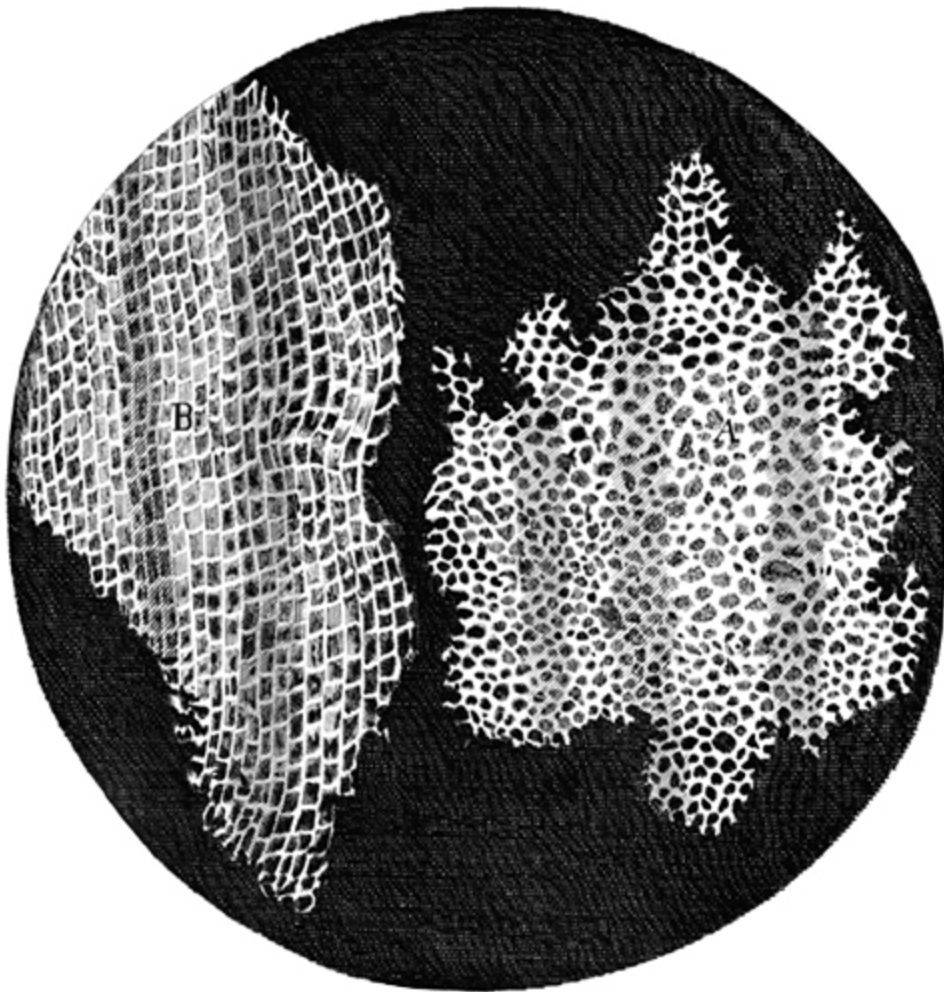
- All cells only come from other cells (the principle of biogenesis).
- Cells are the fundamental units of organisms.

Today, these tenets are fundamental to our understanding of life on earth. However, modern cell theory grew out of the collective work of many scientists.

The Origins of Cell Theory

The English scientist Robert Hooke first used the term “cells” in 1665 to describe the small chambers within cork that he observed under a microscope of his own design. To Hooke, thin sections of cork resembled

“Honey-comb,” or “small Boxes or Bladders of Air.” He noted that each “Cavern, Bubble, or Cell” was distinct from the others ([\[link\]](#)). At the time, Hooke was not aware that the cork cells were long dead and, therefore, lacked the internal structures found within living cells.



Robert Hooke (1635–1703) was the first to describe cells based upon his microscopic observations of cork. This illustration was published in his work *Micrographia*.

Despite Hooke’s early description of cells, their significance as the fundamental unit of life was not yet recognized. Nearly 200 years later, in

1838, Matthias Schleiden (1804–1881), a German botanist who made extensive microscopic observations of plant tissues, described them as being composed of cells. Visualizing plant cells was relatively easy because plant cells are clearly separated by their thick cell walls. Schleiden believed that cells formed through crystallization, rather than cell division.

Theodor Schwann (1810–1882), a noted German physiologist, made similar microscopic observations of animal tissue. In 1839, after a conversation with Schleiden, Schwann realized that similarities existed between plant and animal tissues. This laid the foundation for the idea that cells are the fundamental components of plants and animals.

In the 1850s, two Polish scientists living in Germany pushed this idea further, culminating in what we recognize today as the modern cell theory. In 1852, Robert Remak (1815–1865), a prominent neurologist and embryologist, published convincing evidence that cells are derived from other cells as a result of cell division. However, this idea was questioned by many in the scientific community. Three years later, Rudolf Virchow (1821–1902), a well-respected pathologist, published an editorial essay entitled “Cellular Pathology,” which popularized the concept of cell theory using the Latin phrase *omnis cellula a cellula* (“all cells arise from cells”), which is essentially the second tenet of modern cell theory.[\[footnote\]](#) Given the similarity of Virchow’s work to Remak’s, there is some controversy as to which scientist should receive credit for articulating cell theory. See the following Eye on Ethics feature for more about this controversy.

M. Schultz. “Rudolph Virchow.” *Emerging Infectious Diseases* 14 no. 9 (2008):1480–1481.

Note:**Science and Plagiarism**

Rudolf Virchow, a prominent, Polish-born, German scientist, is often remembered as the “Father of Pathology.” Well known for innovative approaches, he was one of the first to determine the causes of various diseases by examining their effects on tissues and organs. He was also among the first to use animals in his research and, as a result of his work, he was the first to name numerous diseases and created many other

medical terms. Over the course of his career, he published more than 2,000 papers and headed various important medical facilities, including the Charité – Universitätsmedizin Berlin, a prominent Berlin hospital and medical school. But he is, perhaps, best remembered for his 1855 editorial essay titled “Cellular Pathology,” published in *Archiv für Pathologische Anatomie und Physiologie*, a journal that Virchow himself cofounded and still exists today.

Despite his significant scientific legacy, there is some controversy regarding this essay, in which Virchow proposed the central tenet of modern cell theory—that all cells arise from other cells. Robert Remak, a former colleague who worked in the same laboratory as Virchow at the University of Berlin, had published the same idea 3 years before. Though it appears Virchow was familiar with Remak’s work, he neglected to credit Remak’s ideas in his essay. When Remak wrote a letter to Virchow pointing out similarities between Virchow’s ideas and his own, Virchow was dismissive. In 1858, in the preface to one of his books, Virchow wrote that his 1855 publication was just an editorial piece, not a scientific paper, and thus there was no need to cite Remak’s work.

By today’s standards, Virchow’s editorial piece would certainly be considered an act of plagiarism, since he presented Remak’s ideas as his own. However, in the 19th century, standards for academic integrity were much less clear. Virchow’s strong reputation, coupled with the fact that Remak was a Jew in a somewhat anti-Semitic political climate, shielded him from any significant repercussions. Today, the process of peer review and the ease of access to the scientific literature help discourage plagiarism. Although scientists are still motivated to publish original ideas that advance scientific knowledge, those who would consider plagiarizing are well aware of the serious consequences.

In academia, plagiarism represents the theft of both individual thought and research—an offense that can destroy reputations and end careers.

[\[footnote\]](#) [\[footnote\]](#) [\[footnote\]](#) [\[footnote\]](#)

B. Kisch. “Forgotten Leaders in Modern Medicine, Valentin, Gouby, Remak, Auerbach.” *Transactions of the American Philosophical Society* 44 (1954):139–317.

H. Harris. *The Birth of the Cell*. New Haven, CT: Yale University Press, 2000:133.

C. Webster (ed.). *Biology, Medicine and Society 1840-1940*. Cambridge, UK; Cambridge University Press, 1981:118–119.

C. Zuchora-Walske. *Key Discoveries in Life Science*. Minneapolis, MN: Lerner Publishing, 2015:12–13.



(a)



(b)

(a) Rudolf Virchow (1821–1902) popularized the cell theory in an 1855 essay entitled “Cellular Pathology.” (b) The idea that all cells originate from other cells was first published in 1852 by his contemporary and former colleague Robert Remak (1815–1865).

Note:

- What are the key points of the cell theory?
- What contributions did Rudolf Virchow and Robert Remak make to the development of the cell theory?

Endosymbiotic Theory

As scientists were making progress toward understanding the role of cells in plant and animal tissues, others were examining the structures within the cells themselves. In 1831, Scottish botanist Robert Brown (1773–1858) was the first to describe observations of nuclei, which he observed in plant cells. Then, in the early 1880s, German botanist Andreas Schimper (1856–1901) was the first to describe the chloroplasts of plant cells, identifying their role in starch formation during photosynthesis and noting that they divided independent of the nucleus.

Based upon the chloroplasts' ability to reproduce independently, Russian botanist Konstantin Mereschkowski (1855–1921) suggested in 1905 that chloroplasts may have originated from ancestral photosynthetic bacteria living symbiotically inside a eukaryotic cell. He proposed a similar origin for the nucleus of plant cells. This was the first articulation of the endosymbiotic hypothesis, and would explain how eukaryotic cells evolved from ancestral bacteria.

Mereschkowski's endosymbiotic hypothesis was furthered by American anatomist Ivan Wallin (1883–1969), who began to experimentally examine the similarities between mitochondria, chloroplasts, and bacteria—in other words, to put the endosymbiotic hypothesis to the test using objective investigation. Wallin published a series of papers in the 1920s supporting the endosymbiotic hypothesis, including a 1926 publication co-authored with Mereschkowski. Wallin claimed he could culture mitochondria outside of their eukaryotic host cells. Many scientists dismissed his cultures of mitochondria as resulting from bacterial contamination. Modern genome sequencing work supports the dissenting scientists by showing that much of the genome of mitochondria had been transferred to the host cell's nucleus, preventing the mitochondria from being able to live on their own. [\[footnote\]](#) [\[footnote\]](#)

T. Embley, W. Martin. "Eukaryotic Evolution, Changes, and Challenges." *Nature* Vol. 440 (2006):623–630.

O.G. Berg, C.G. Kurland. "Why Mitochondrial Genes Are Most Often Found in Nuclei." *Molecular Biology and Evolution* 17 no. 6 (2000):951–961.

Wallin's ideas regarding the endosymbiotic hypothesis were largely ignored for the next 50 years because scientists were unaware that these organelles contained their own DNA. However, with the discovery of mitochondrial and chloroplast DNA in the 1960s, the endosymbiotic hypothesis was resurrected. Lynn Margulis (1938–2011), an American geneticist, published her ideas regarding the endosymbiotic hypothesis of the origins of mitochondria and chloroplasts in 1967.[\[footnote\]](#) In the decade leading up to her publication, advances in microscopy had allowed scientists to differentiate prokaryotic cells from eukaryotic cells. In her publication, Margulis reviewed the literature and argued that the eukaryotic organelles such as mitochondria and chloroplasts are of prokaryotic origin. She presented a growing body of microscopic, genetic, molecular biology, fossil, and geological data to support her claims.

L. Sagan. "On the Origin of Mitosing Cells." *Journal of Theoretical Biology* 14 no. 3 (1967):225–274.

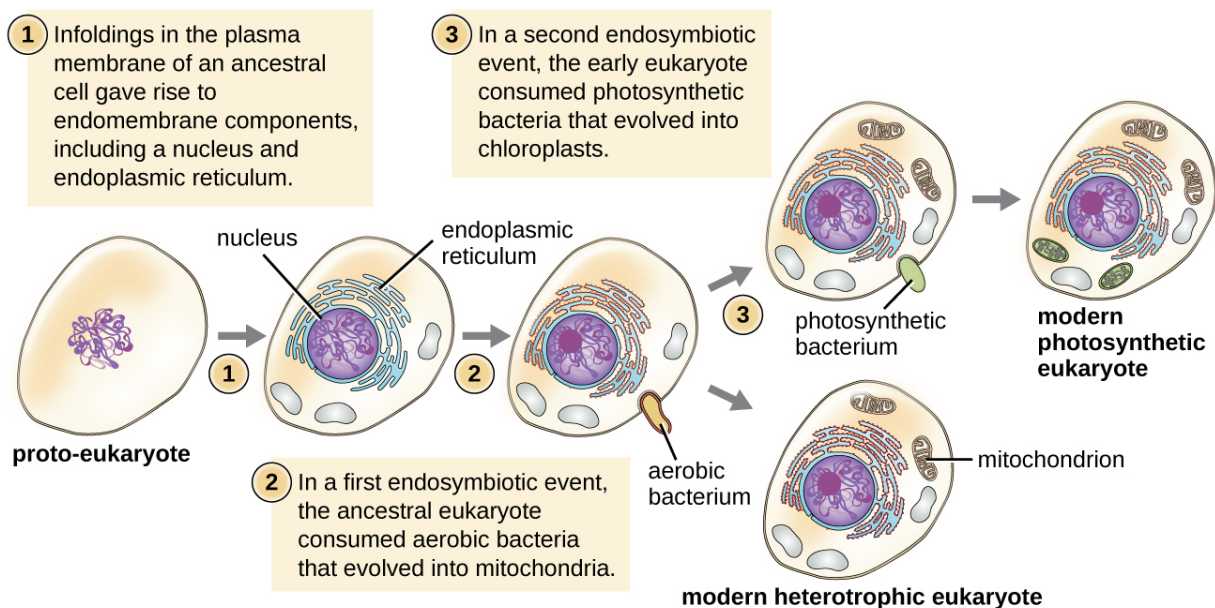
Again, this hypothesis was not initially popular, but mounting genetic evidence due to the advent of DNA sequencing supported the **endosymbiotic theory**, which is now defined as the theory that mitochondria and chloroplasts arose as a result of prokaryotic cells establishing a symbiotic relationship within a eukaryotic host ([\[link\]](#)). With Margulis' initial endosymbiotic theory gaining wide acceptance, she expanded on the theory in her 1981 book *Symbiosis in Cell Evolution*. In it, she explains how endosymbiosis is a major driving factor in the evolution of organisms. More recent genetic sequencing and phylogenetic analysis show that mitochondrial DNA and chloroplast DNA are highly related to their bacterial counterparts, both in DNA sequence and chromosome structure. However, mitochondrial DNA and chloroplast DNA are reduced compared with nuclear DNA because many of the genes have moved from the organelles into the host cell's nucleus. Additionally, mitochondrial and chloroplast ribosomes are structurally similar to bacterial ribosomes, rather than to the eukaryotic ribosomes of their hosts. Last, the binary fission of these organelles strongly resembles the binary fission of bacteria, as compared with mitosis performed by eukaryotic cells. Since Margulis' original proposal, scientists have observed several examples of bacterial endosymbionts in modern-day eukaryotic cells. Examples include the endosymbiotic bacteria found within the guts of certain insects, such as

cockroaches,[\[footnote\]](#) and photosynthetic bacteria-like organelles found in protists.[\[footnote\]](#)

A.E. Douglas. “The Microbial Dimension in Insect Nutritional Ecology.” *Functional Ecology* 23 (2009):38–47.

J.M. Jaynes, L.P. Vernon. “The Cyanelle of *Cyanophora paradoxa*: Almost a Cyanobacterial Chloroplast.” *Trends in Biochemical Sciences* 7 no. 1 (1982):22–24.

The Endosymbiotic Theory



According to the endosymbiotic theory, mitochondria and chloroplasts are each derived from the uptake of bacteria. These bacteria established a symbiotic relationship with their host cell that eventually led to the bacteria evolving into mitochondria and chloroplasts.

Note:

- What does the modern endosymbiotic theory state?

- What evidence supports the endosymbiotic theory?

The Germ Theory of Disease

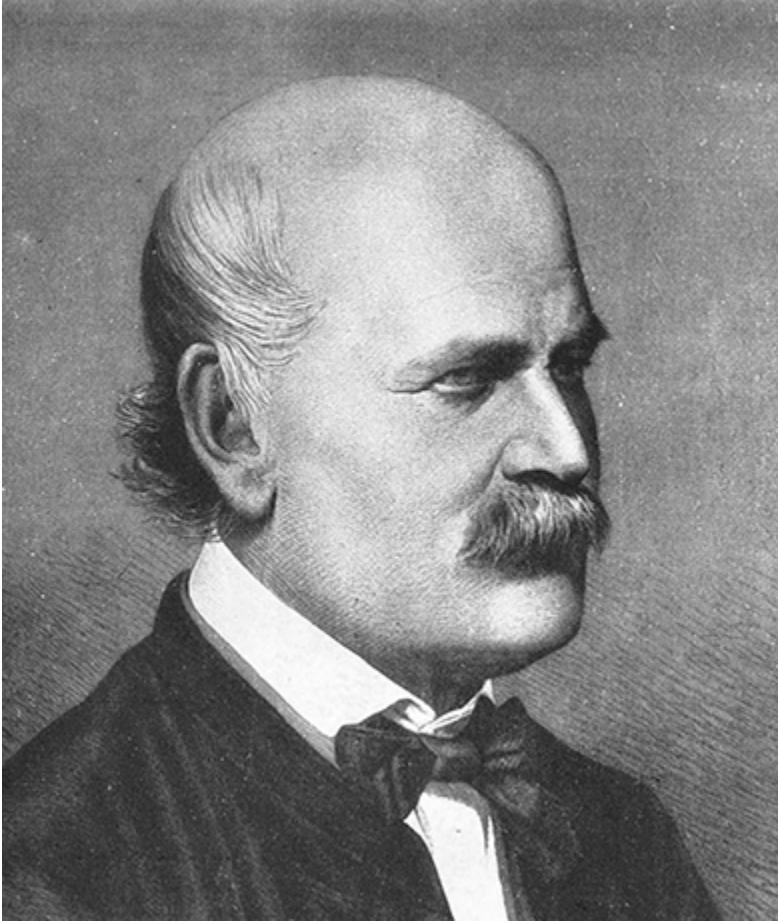
Prior to the discovery of microbes during the 17th century, other theories circulated about the origins of disease. For example, the ancient Greeks proposed the miasma theory, which held that disease originated from particles emanating from decomposing matter, such as that in sewage or cesspits. Such particles infected humans in close proximity to the rotting material. Diseases including the Black Death, which ravaged Europe's population during the Middle Ages, were thought to have originated in this way.

In 1546, Italian physician Girolamo Fracastoro proposed, in his essay *De Contagione et Contagiosis Morbis*, that seed-like spores may be transferred between individuals through direct contact, exposure to contaminated clothing, or through the air. We now recognize Fracastoro as an early proponent of the **germ theory of disease**, which states that diseases may result from microbial infection. However, in the 16th century, Fracastoro's ideas were not widely accepted and would be largely forgotten until the 19th century.

In 1847, Hungarian obstetrician Ignaz Semmelweis ([link](#)) observed that mothers who gave birth in hospital wards staffed by physicians and medical students were more likely to suffer and die from puerperal fever after childbirth (10%–20% mortality rate) than were mothers in wards staffed by midwives (1% mortality rate). Semmelweis observed medical students performing autopsies and then subsequently carrying out vaginal examinations on living patients without washing their hands in between. He suspected that the students carried disease from the autopsies to the patients they examined. His suspicions were supported by the untimely death of a friend, a physician who contracted a fatal wound infection after a postmortem examination of a woman who had died of a puerperal infection. The dead physician's wound had been caused by a scalpel used during the

examination, and his subsequent illness and death closely paralleled that of the dead patient.

Although Semmelweis did not know the true cause of puerperal fever, he proposed that physicians were somehow transferring the causative agent to their patients. He suggested that the number of puerperal fever cases could be reduced if physicians and medical students simply washed their hands with chlorinated lime water before and after examining every patient. When this practice was implemented, the maternal mortality rate in mothers cared for by physicians dropped to the same 1% mortality rate observed among mothers cared for by midwives. This demonstrated that handwashing was a very effective method for preventing disease transmission. Despite this great success, many discounted Semmelweis's work at the time, and physicians were slow to adopt the simple procedure of handwashing to prevent infections in their patients because it contradicted established norms for that time period.



Ignaz Semmelweis (1818–1865) was a proponent of the importance of handwashing to prevent transfer of disease between patients by physicians.

Around the same time Semmelweis was promoting handwashing, in 1848, British physician John Snow conducted studies to track the source of cholera outbreaks in London. By tracing the outbreaks to two specific water sources, both of which were contaminated by sewage, Snow ultimately demonstrated that cholera bacteria were transmitted via drinking water. Snow's work is influential in that it represents the first known epidemiological study, and it resulted in the first known public health response to an epidemic. The work of both Semmelweis and Snow clearly

refuted the prevailing miasma theory of the day, showing that disease is not only transmitted through the air but also through contaminated items.

Although the work of Semmelweis and Snow successfully showed the role of sanitation in preventing infectious disease, the cause of disease was not fully understood. The subsequent work of Louis Pasteur, Robert Koch, and Joseph Lister would further substantiate the germ theory of disease.

While studying the causes of beer and wine spoilage in 1856, Pasteur discovered properties of fermentation by microorganisms. He had demonstrated with his swan-neck flask experiments ([\[link\]](#)) that airborne microbes, not spontaneous generation, were the cause of food spoilage, and he suggested that if microbes were responsible for food spoilage and fermentation, they could also be responsible for causing infection. This was the foundation for the germ theory of disease.

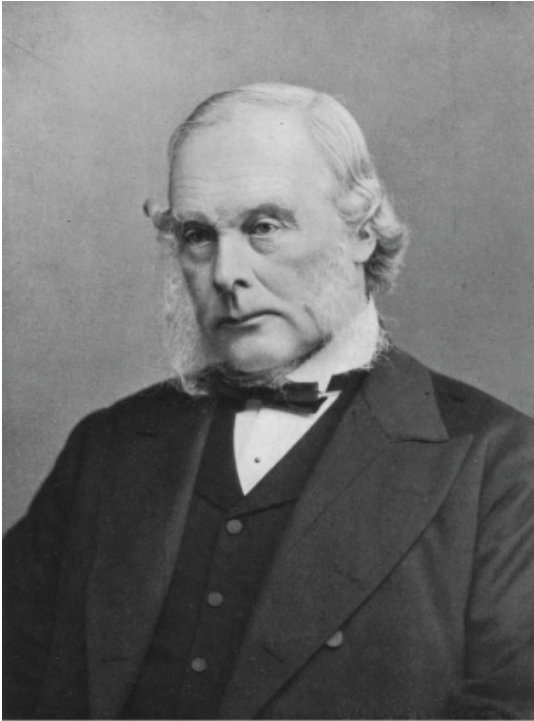
Meanwhile, British surgeon Joseph Lister ([\[link\]](#)) was trying to determine the causes of postsurgical infections. Many physicians did not give credence to the idea that microbes on their hands, on their clothes, or in the air could infect patients' surgical wounds, despite the fact that 50% of surgical patients, on average, were dying of postsurgical infections.

[\[footnote\]](#) Lister, however, was familiar with the work of Semmelweis and Pasteur; therefore, he insisted on handwashing and extreme cleanliness during surgery. In 1867, to further decrease the incidence of postsurgical wound infections, Lister began using carbolic acid (phenol) spray disinfectant/antiseptic during surgery. His extremely successful efforts to reduce postsurgical infection caused his techniques to become a standard medical practice.

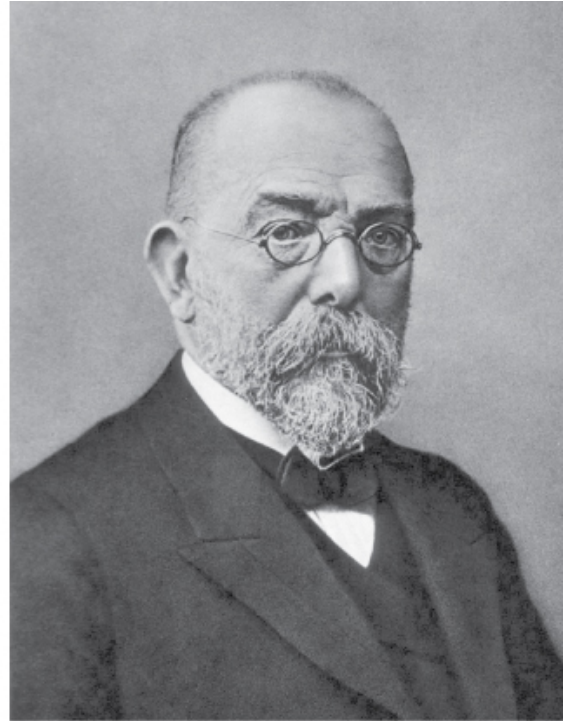
Alexander, J. Wesley. "The Contributions of Infection Control to a Century of Progress" *Annals of Surgery* 201:423-428, 1985.

A few years later, Robert Koch ([\[link\]](#)) proposed a series of postulates (Koch's postulates) based on the idea that the cause of a specific disease could be attributed to a specific microbe. Using these postulates, Koch and his colleagues were able to definitively identify the causative pathogens of specific diseases, including anthrax, tuberculosis, and cholera. Koch's "one microbe, one disease" concept was the culmination of the 19th century's paradigm shift away from miasma theory and toward the germ theory of

disease. Koch's postulates are discussed more thoroughly in [How Pathogens Cause Disease](#).



(a)



(b)

(a) Joseph Lister developed procedures for the proper care of surgical wounds and the sterilization of surgical equipment. (b) Robert Koch established a protocol to determine the cause of infectious disease. Both scientists contributed significantly to the acceptance of the germ theory of disease.

Note:

- Compare and contrast the miasma theory of disease with the germ theory of disease.

- How did Joseph Lister’s work contribute to the debate between the miasma theory and germ theory and how did this increase the success of medical procedures?

Note:

Part 2

After suffering a fever, congestion, cough, and increasing aches and pains for several days, Barbara suspects that she has a case of the flu. She decides to visit the health center at her university. The PA tells Barbara that her symptoms could be due to a range of diseases, such as influenza, bronchitis, pneumonia, or tuberculosis.

During her physical examination, the PA notes that Barbara’s heart rate is slightly elevated. Using a pulse oximeter, a small device that clips on her finger, he finds that Barbara has hypoxemia—a lower-than-normal level of oxygen in the blood. Using a stethoscope, the PA listens for abnormal sounds made by Barbara’s heart, lungs, and digestive system. As Barbara breathes, the PA hears a crackling sound and notes a slight shortness of breath. He collects a sputum sample, noting the greenish color of the mucus, and orders a chest radiograph, which shows a “shadow” in the left lung. All of these signs are suggestive of pneumonia, a condition in which the lungs fill with mucus ([\[link\]](#)).



lung infiltrated, suggestive of pneumonia

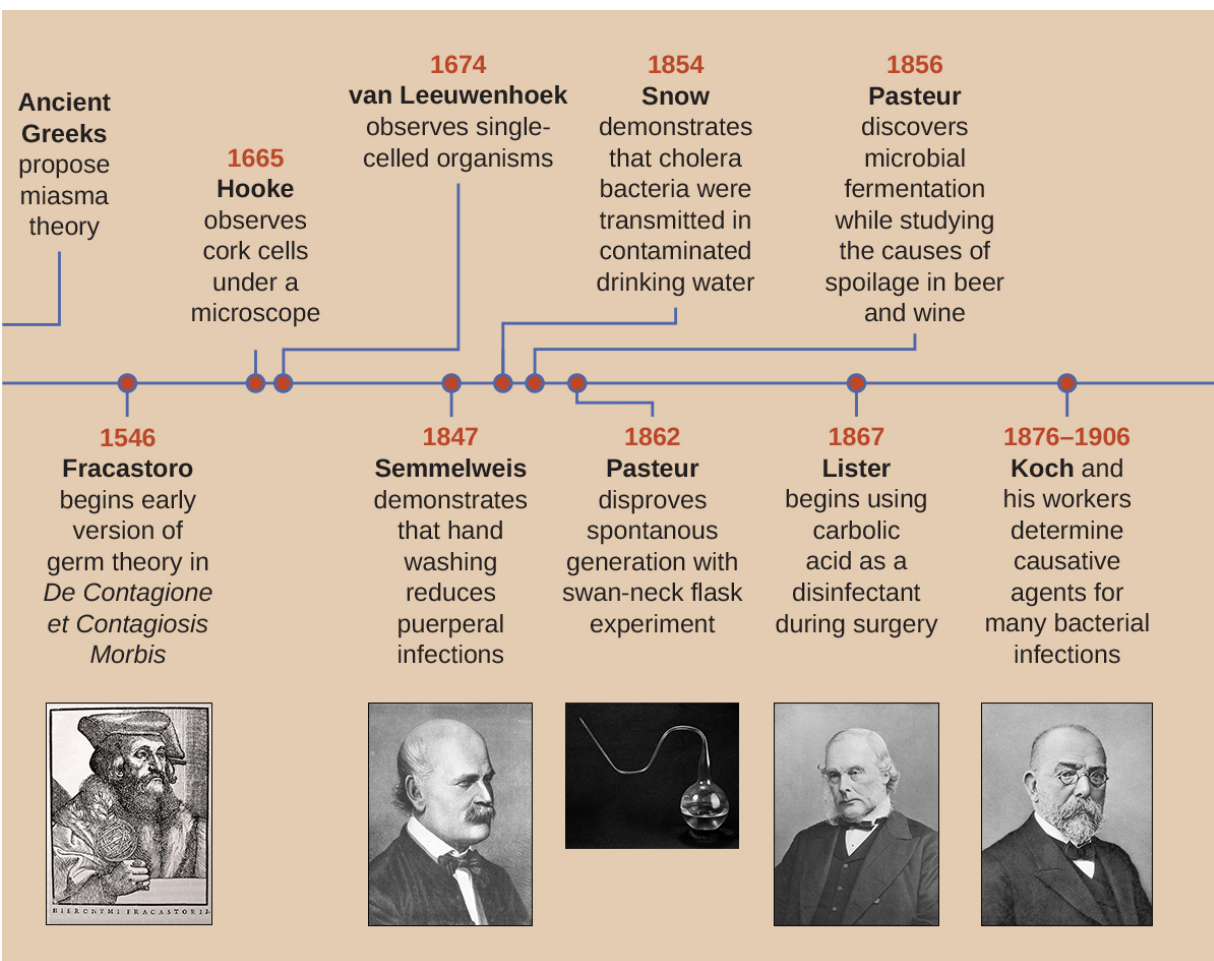


normal lungs

This is a chest radiograph typical of pneumonia. Because X-ray images are negative images, a “shadow” is seen as a white area within the lung that should otherwise be black. In this case, the left lung shows a shadow as a result of pockets in the lung that have become filled with fluid. (credit left: modification of work by “Christaras A”/Wikimedia Commons)

- What kinds of infectious agents are known to cause pneumonia?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.



(credit “swan-neck flask”: modification of work by Wellcome Images)

Key Concepts and Summary

- Although cells were first observed in the 1660s by Robert Hooke, **cell theory** was not well accepted for another 200 years. The work of scientists such as Schleiden, Schwann, Remak, and Virchow contributed to its acceptance.
- **Endosymbiotic theory** states that mitochondria and chloroplasts, organelles found in many types of organisms, have their origins in bacteria. Significant structural and genetic information support this theory.
- The **miasma theory of disease** was widely accepted until the 19th century, when it was replaced by the **germ theory of disease** thanks to the work of Semmelweis, Snow, Pasteur, Lister, and Koch, and others.

Multiple Choice

Exercise:

Problem:

Which of the following individuals did not contribute to the establishment of cell theory?

- A. Girolamo Fracastoro
- B. Matthias Schleiden
- C. Robert Remak
- D. Robert Hooke

Solution:

A

Exercise:

Problem:

Whose proposal of the endosymbiotic theory of mitochondrial and chloroplast origin was ultimately accepted by the greater scientific community?

- A. Rudolf Virchow
- B. Ignaz Semmelweis
- C. Lynn Margulis
- D. Theodor Schwann

Solution:

C

Exercise:

Problem:

Which of the following developed a set of postulates for determining whether a particular disease is caused by a particular pathogen?

- A. John Snow
- B. Robert Koch
- C. Joseph Lister
- D. Louis Pasteur

Solution:

B

Fill in the Blank

Exercise:

Problem: John Snow is known as the Father of _____.

Solution:

epidemiology

Exercise:

Problem:

The _____ theory states that disease may originate from proximity to decomposing matter and is not due to person-to-person contact.

Solution:

miasma

Exercise:

Problem: The scientist who first described cells was _____.

Solution:

Robert Hooke

Short Answer

Exercise:

Problem:

How did the explanation of Virchow and Remak for the origin of cells differ from that of Schleiden and Schwann?

Exercise:

Problem:

What evidence exists that supports the endosymbiotic theory?

Exercise:**Problem:**

What were the differences in mortality rates due to puerperal fever that Ignaz Semmelweis observed? How did he propose to reduce the occurrence of puerperal fever? Did it work?

Critical Thinking**Exercise:****Problem:**

Why are mitochondria and chloroplasts unable to multiply outside of a host cell?

Exercise:**Problem:**

Why was the work of Snow so important in supporting the germ theory?

Unique Characteristics of Prokaryotic Cells

LEARNING OBJECTIVES

- Explain the distinguishing characteristics of prokaryotic cells
- Describe common cell morphologies and cellular arrangements typical of prokaryotic cells and explain how cells maintain their morphology
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Compare the distinguishing characteristics of bacterial and archaeal cells

Cell theory states that the cell is the fundamental unit of life. However, cells vary significantly in size, shape, structure, and function. At the simplest level of construction, all cells possess a few fundamental components. These include **cytoplasm** (a gel-like substance composed of water and dissolved chemicals needed for growth), which is contained within a plasma membrane (also called a cell membrane or cytoplasmic membrane); one or more chromosomes, which contain the genetic blueprints of the cell; and **ribosomes**, organelles used for the production of proteins.

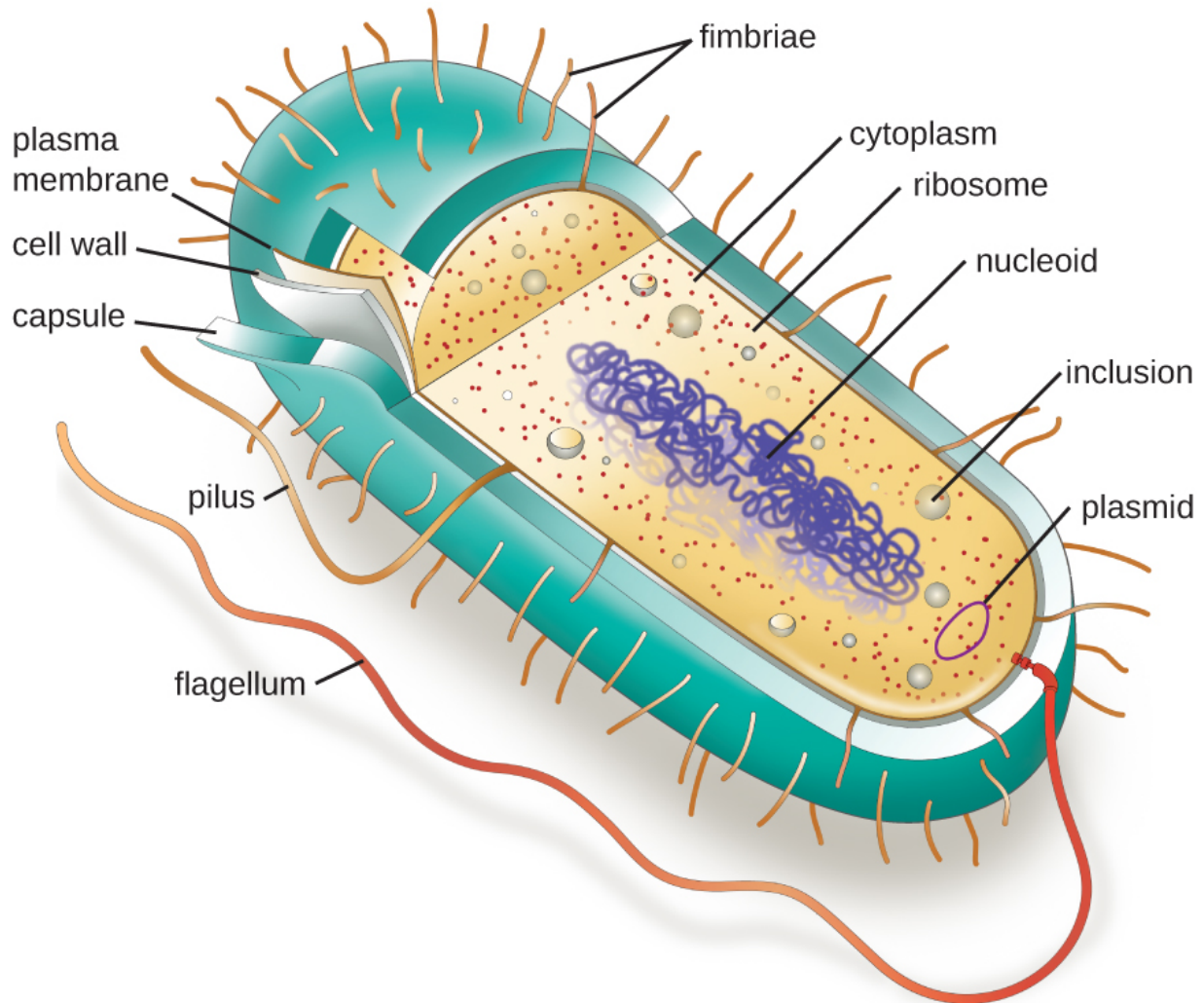
Beyond these basic components, cells can vary greatly between organisms, and even within the same multicellular organism. The two largest categories of cells—**prokaryotic cells** and **eukaryotic cells**—are defined by major differences in several cell structures. Prokaryotic cells lack a nucleus surrounded by a complex nuclear membrane and generally have a single, circular chromosome located in a nucleoid. Eukaryotic cells have a nucleus

surrounded by a complex nuclear membrane that contains multiple, rod-shaped chromosomes.[\[footnote\]](#)

Y.-H.M. Chan, W.F. Marshall. “Scaling Properties of Cell and Organelle Size.” *Organogenesis* 6 no. 2 (2010):88–96.

All plant cells and animal cells are eukaryotic. Some microorganisms are composed of prokaryotic cells, whereas others are composed of eukaryotic cells. Prokaryotic microorganisms are classified within the domains Archaea and Bacteria, whereas eukaryotic organisms are classified within the domain Eukarya.

The structures inside a cell are analogous to the organs inside a human body, with unique structures suited to specific functions. Some of the structures found in prokaryotic cells are similar to those found in some eukaryotic cells; others are unique to prokaryotes. Although there are some exceptions, eukaryotic cells tend to be larger than prokaryotic cells. The comparatively larger size of eukaryotic cells dictates the need to compartmentalize various chemical processes within different areas of the cell, using complex membrane-bound organelles. In contrast, prokaryotic cells generally lack membrane-bound organelles; however, they often contain inclusions that compartmentalize their cytoplasm. [\[link\]](#) illustrates structures typically associated with prokaryotic cells. These structures are described in more detail in the next section.

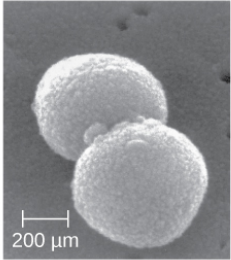
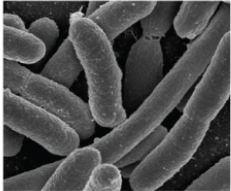
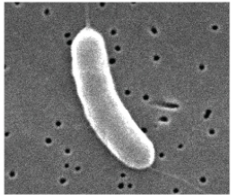
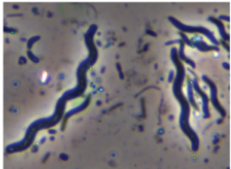


A typical prokaryotic cell contains a cell membrane, chromosomal DNA that is concentrated in a nucleoid, ribosomes, and a cell wall. Some prokaryotic cells may also possess flagella, pili, fimbriae, and capsules.

Common Cell Morphologies and Arrangements

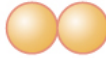
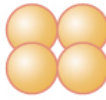
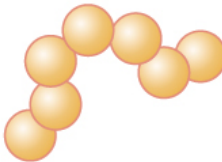
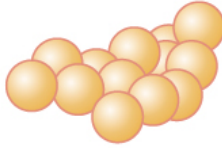
Individual cells of a particular prokaryotic organism are typically similar in shape, or **cell morphology**. Although thousands of prokaryotic organisms have been identified, only a handful of cell morphologies are commonly seen microscopically. [\[link\]](#) names and illustrates cell morphologies

commonly found in prokaryotic cells. In addition to cellular shape, prokaryotic cells of the same species may group together in certain distinctive arrangements depending on the plane of cell division. Some common arrangements are shown in [\[link\]](#).

Common Prokaryotic Cell Shapes			
Name	Description	Illustration	Image
Coccus (pl. cocci)	Round		
Bacillus (pl. bacilli)	Rod		
Vibrio (pl. vibrios)	Curved rod		
Coccobacillus (pl. coccobacilli)	Short rod		
Spirillum (pl. spirilla)	Spiral		
Spirochete (pl. spirochetes)	Long, loose, helical spiral		

(credit “Coccus” micrograph: modification of work by Janice Haney

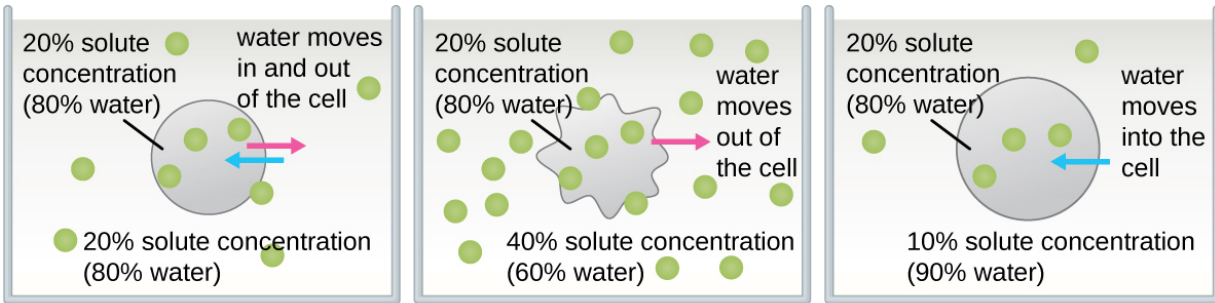
Carr, Centers for Disease Control and Prevention; credit
 “Coccobacillus” micrograph: modification of work by Janice Carr,
 Centers for Disease Control and Prevention; credit “Spirochete”
 micrograph: modification of work by Centers for Disease Control and
 Prevention)

Common Prokaryotic Cell Arrangements		
Name	Description	Illustration
Coccus (pl. cocci)	Single coccus	
Diplococcus (pl. diplococci)	Pair of two cocci	
Tetrad (pl. tetrads)	Grouping of four cells arranged in a square	
Streptococcus (pl. streptococci)	Chain of cocci	
Staphylococcus (pl. staphylococci)	Cluster of cocci	
Bacillus (pl. bacilli)	Single rod	
Streptobacillus (pl. streptobacilli)	Chain of rods	

In most prokaryotic cells, morphology is maintained by the **cell wall** in combination with cytoskeletal elements. The cell wall is a structure found in most prokaryotes and some eukaryotes; it envelopes the cell membrane, protecting the cell from changes in **osmotic pressure** ([\[link\]](#)). Osmotic pressure occurs because of differences in the concentration of solutes on opposing sides of a semipermeable membrane. Water is able to pass through a semipermeable membrane, but solutes (dissolved molecules like salts, sugars, and other compounds) cannot. When the concentration of solutes is greater on one side of the membrane, water diffuses across the membrane from the side with the lower concentration (more water) to the side with the higher concentration (less water) until the concentrations on both sides become equal. This diffusion of water is called **osmosis**, and it can cause extreme osmotic pressure on a cell when its external environment changes.

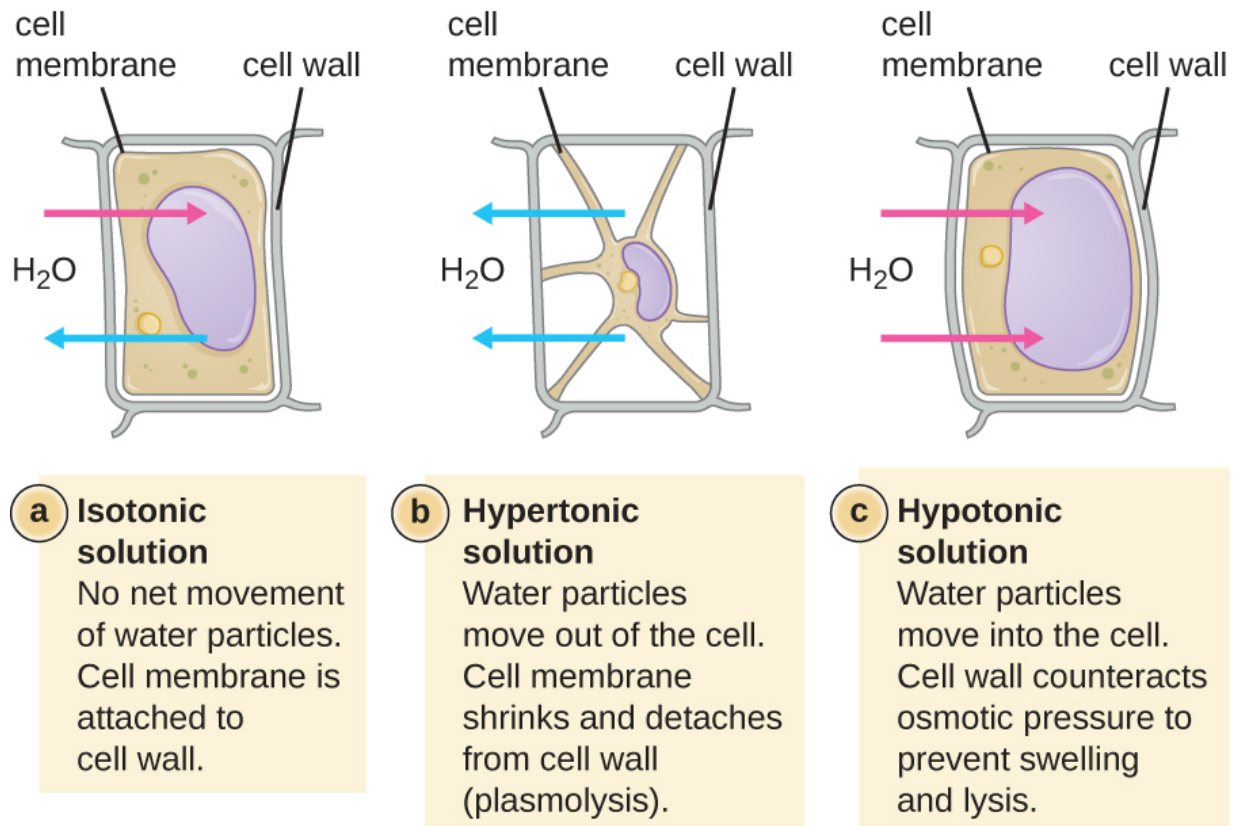
The external environment of a cell can be described as an isotonic, hypertonic, or hypotonic medium. In an **isotonic medium**, the solute concentrations inside and outside the cell are approximately equal, so there is no net movement of water across the cell membrane. In a **hypertonic medium**, the solute concentration outside the cell exceeds that inside the cell, so water diffuses out of the cell and into the external medium. In a **hypotonic medium**, the solute concentration inside the cell exceeds that outside of the cell, so water will move by osmosis into the cell. This causes the cell to swell and potentially lyse, or burst.

The degree to which a particular cell is able to withstand changes in osmotic pressure is called tonicity. Cells that have a cell wall are better able to withstand subtle changes in osmotic pressure and maintain their shape. In hypertonic environments, cells that lack a cell wall can become dehydrated, causing **crenation**, or shriveling of the cell; the plasma membrane contracts and appears scalloped or notched ([\[link\]](#)). By contrast, cells that possess a cell wall undergo **plasmolysis** rather than crenation. In plasmolysis, the plasma membrane contracts and detaches from the cell wall, and there is a decrease in interior volume, but the cell wall remains intact, thus allowing the cell to maintain some shape and integrity for a period of time ([\[link\]](#)). Likewise, cells that lack a cell wall are more prone to lysis in hypotonic environments. The presence of a cell wall allows the cell to maintain its shape and integrity for a longer time before lysing ([\[link\]](#)).



- a Isotonic solution**
A solution that has the *same* solute concentration as another solution. There is no net movement of water particles, and the overall concentration on both sides of the cell membrane remains constant.
- b Hypertonic solution**
A solution that has a *higher* solute concentration than another solution. Water particles will move out of the cell, causing crenation.
- c Hypotonic solution**
A solution that has a *lower* solute concentration than another solution. Water particles will move into the cell, causing the cell to expand and eventually lyse.

In cells that lack a cell wall, changes in osmotic pressure can lead to crenation in hypertonic environments or cell lysis in hypotonic environments.



In prokaryotic cells, the cell wall provides some protection against changes in osmotic pressure, allowing it to maintain its shape longer. The cell membrane is typically attached to the cell wall in an isotonic medium (left). In a hypertonic medium, the cell membrane detaches from the cell wall and contracts (plasmolysis) as water leaves the cell.

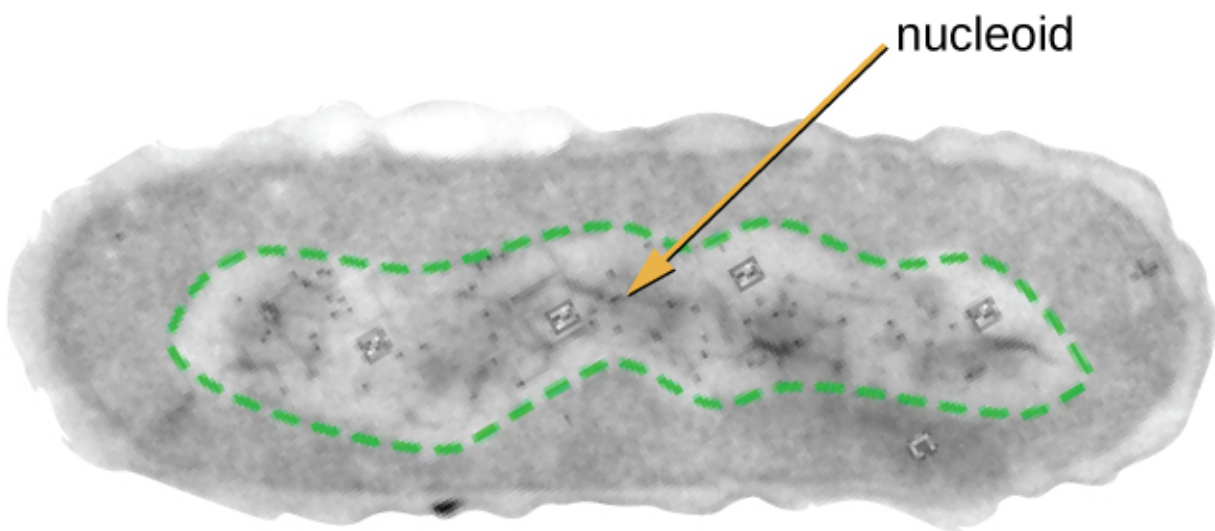
In a hypotonic medium (right), the cell wall prevents the cell membrane from expanding to the point of bursting, although lysis will eventually occur if too much water is absorbed.

Note:

- Explain the difference between cell morphology and arrangement.
- What advantages do cell walls provide prokaryotic cells?

The Nucleoid

All cellular life has a DNA genome organized into one or more chromosomes. Prokaryotic chromosomes are typically circular, haploid (unpaired), and not bound by a complex nuclear membrane. Prokaryotic DNA and DNA-associated proteins are concentrated within the **nucleoid** region of the cell ([\[link\]](#)). In general, prokaryotic DNA interacts with **nucleoid-associated proteins (NAPs)** that assist in the organization and packaging of the chromosome. In bacteria, NAPs function similar to histones, which are the DNA-organizing proteins found in eukaryotic cells. In archaea, the nucleoid is organized by either NAPs or histone-like DNA organizing proteins.



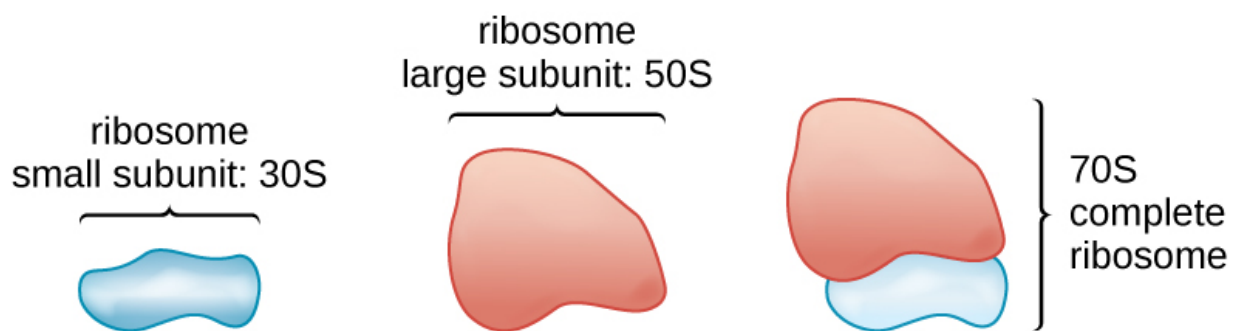
The nucleoid region (the area enclosed by the green dashed line) is a condensed area of DNA found within prokaryotic cells. Because of the density of the area, it does not readily stain and appears lighter in color when viewed with a transmission electron microscope.

Plasmids

Prokaryotic cells may also contain extrachromosomal DNA, or DNA that is not part of the chromosome. This extrachromosomal DNA is found in **plasmids**, which are small, circular, double-stranded DNA molecules. Cells that have plasmids often have hundreds of them within a single cell. Plasmids are more commonly found in bacteria; however, plasmids have been found in archaea and eukaryotic organisms. Plasmids often carry genes that confer advantageous traits such as antibiotic resistance; thus, they are important to the survival of the organism. We will discuss plasmids in more detail in [Mechanisms of Microbial Genetics](#).

Ribosomes

All cellular life synthesizes proteins, and organisms in all three domains of life possess ribosomes, structures responsible for protein synthesis. However, ribosomes in each of the three domains are structurally different. Ribosomes, themselves, are constructed from proteins, along with ribosomal RNA (rRNA). Prokaryotic ribosomes are found in the cytoplasm. They are called **70S ribosomes** because they have a size of 70S ([\[link\]](#)), whereas eukaryotic cytoplasmic ribosomes have a size of 80S. (The S stands for Svedberg unit, a measure of sedimentation in an ultracentrifuge, which is based on size, shape, and surface qualities of the structure being analyzed). Although they are the same size, bacterial and archaeal ribosomes have different proteins and rRNA molecules, and the archaeal versions are more similar to their eukaryotic counterparts than to those found in bacteria.



Prokaryotic ribosomes (70S) are composed of two subunits: the 30S

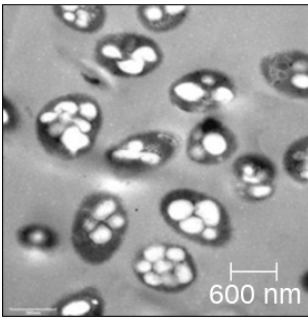
(small subunit) and the 50S (large subunit), each of which are composed of protein and rRNA components.

Inclusions

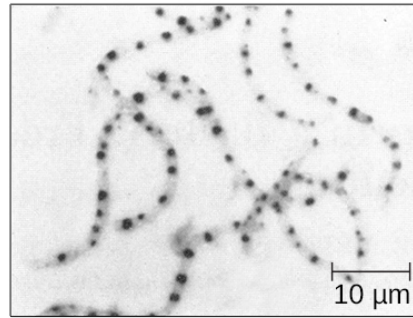
As single-celled organisms living in unstable environments, some prokaryotic cells have the ability to store excess nutrients within cytoplasmic structures called **inclusions**. Storing nutrients in a polymerized form is advantageous because it reduces the buildup of osmotic pressure that occurs as a cell accumulates solutes. Various types of inclusions store glycogen and starches, which contain carbon that cells can access for energy. **Volutin** granules, also called **metachromatic granules** because of their staining characteristics, are inclusions that store polymerized inorganic phosphate that can be used in metabolism and assist in the formation of biofilms. Microbes known to contain volutin granules include the archaea *Methanosarcina*, the bacterium *Corynebacterium diphtheriae*, and the unicellular eukaryotic alga *Chlamydomonas*. Sulfur granules, another type of inclusion, are found in sulfur bacteria of the genus *Thiobacillus*; these granules store elemental sulfur, which the bacteria use for metabolism.

Occasionally, certain types of inclusions are surrounded by a phospholipid monolayer embedded with protein. **Polyhydroxybutyrate (PHB)**, which can be produced by species of *Bacillus* and *Pseudomonas*, is an example of an inclusion that displays this type of monolayer structure. Industrially, PHB has also been used as a source of biodegradable polymers for bioplastics. Several different types of inclusions are shown in [\[link\]](#).

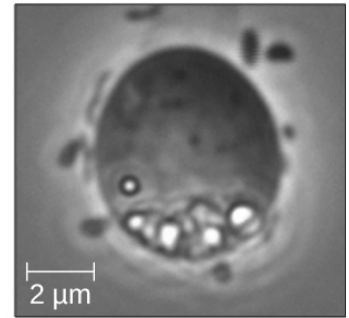
prokaryote inclusion bodies



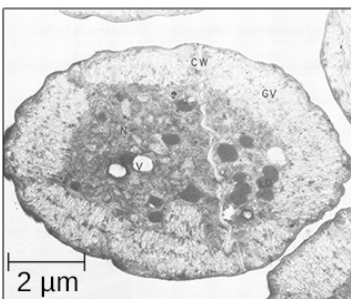
(a)



(b)



(c)



(d)



(e)

Prokaryotic cells may have various types of inclusions. (a) A transmission electron micrograph of polyhydroxybutyrate lipid droplets. (b) A light micrograph of volutin granules. (c) A phase-contrast micrograph of sulfur granules. (d) A transmission electron micrograph of gas vacuoles. (e) A transmission electron micrograph of magnetosomes. (credit b, c, d: modification of work by American Society for Microbiology)

Some prokaryotic cells have other types of inclusions that serve purposes other than nutrient storage. For example, some prokaryotic cells produce gas vacuoles, accumulations of small, protein-lined vesicles of gas. These gas vacuoles allow the prokaryotic cells that synthesize them to alter their buoyancy so that they can adjust their location in the water column. Magnetotactic bacteria, such as *Magnetospirillum magnetotacticum*, contain **magnetosomes**, which are inclusions of magnetic iron oxide or iron

sulfide surrounded by a lipid layer. These allow cells to align along a magnetic field, aiding their movement ([\[link\]](#)). Cyanobacteria such as *Anabaena cylindrica* and bacteria such as *Halothiobacillus neapolitanus* produce **carboxysome** inclusions. Carboxysomes are composed of outer shells of thousands of protein subunits. Their interior is filled with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase. Both of these compounds are used for carbon metabolism. Some prokaryotic cells also possess carboxysomes that sequester functionally related enzymes in one location. These structures are considered proto-organelles because they compartmentalize important compounds or chemical reactions, much like many eukaryotic organelles.

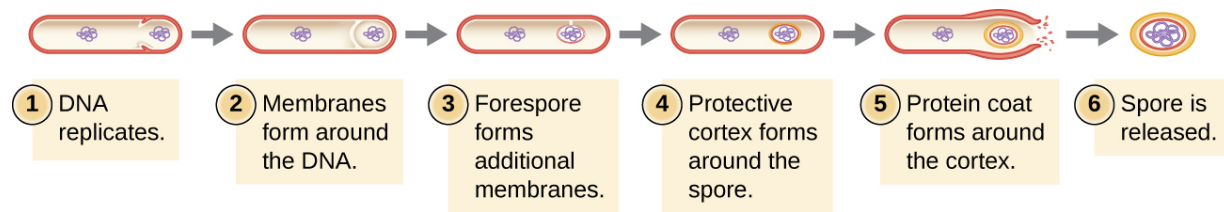
Endospores

Bacterial cells are generally observed as **vegetative cells**, but some genera of bacteria have the ability to form **endospores**, structures that essentially protect the bacterial genome in a dormant state when environmental conditions are unfavorable. Endospores (not to be confused with the reproductive spores formed by fungi) allow some bacterial cells to survive long periods without food or water, as well as exposure to chemicals, extreme temperatures, and even radiation. [\[link\]](#) compares the characteristics of vegetative cells and endospores.

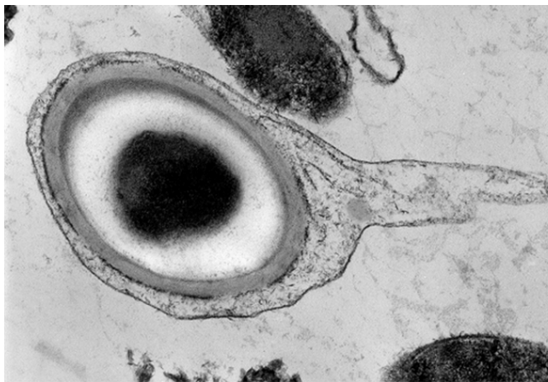
Characteristics of Vegetative Cells versus Endospores	
Vegetative Cells	Endospores
Sensitive to extreme temperatures and radiation	Resistant to extreme temperatures and radiation

Characteristics of Vegetative Cells versus Endospores	
Vegetative Cells	Endospores
Gram-positive	Do not absorb Gram stain, only special endospore stains (see Staining Microscopic Specimens)
Normal water content and enzymatic activity	Dehydrated; no metabolic activity
Capable of active growth and metabolism	Dormant; no growth or metabolic activity

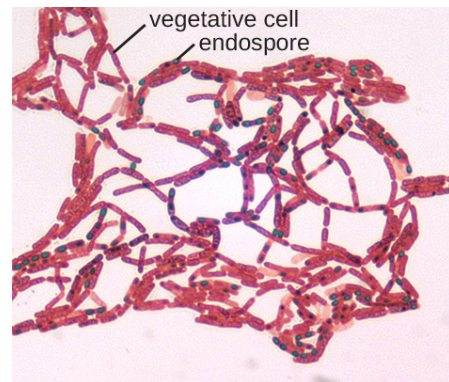
The process by which vegetative cells transform into endospores is called **sporulation**, and it generally begins when nutrients become depleted or environmental conditions become otherwise unfavorable ([\[link\]](#)). The process begins with the formation of a septum in the vegetative bacterial cell. The septum divides the cell asymmetrically, separating a DNA forespore from the mother cell. The forespore, which will form the core of the endospore, is essentially a copy of the cell's chromosomes, and is separated from the mother cell by a second membrane. A cortex gradually forms around the forespore by laying down layers of calcium and dipicolinic acid between membranes. A protein spore coat then forms around the cortex while the DNA of the mother cell disintegrates. Further maturation of the endospore occurs with the formation of an outermost exosporium. The endospore is released upon disintegration of the mother cell, completing sporulation.



(a)



(b)



(c)

(a) Sporulation begins following asymmetric cell division. The forespore becomes surrounded by a double layer of membrane, a cortex, and a protein spore coat, before being released as a mature endospore upon disintegration of the mother cell. (b) An electron micrograph of a *Carboxydothemus hydrogenofomans* endospore. (c) These *Bacillus* spp. cells are undergoing sporulation. The endospores have been visualized using Malachite Green spore stain. (credit b: modification of work by Jonathan Eisen)

Endospores of certain species have been shown to persist in a dormant state for extended periods of time, up to thousands of years.[\[footnote\]](#) However, when living conditions improve, endospores undergo **germination**, reentering a vegetative state. After germination, the cell becomes metabolically active again and is able to carry out all of its normal functions, including growth and cell division.

F. Rothfuss, M Bender, R Conrad. "Survival and Activity of Bacteria in a Deep, Aged Lake Sediment (Lake Constance)." *Microbial Ecology* 33 no. 1 (1997):69–77.

Not all bacteria have the ability to form endospores; however, there are a number of clinically significant endospore-forming gram-positive bacteria of the genera *Bacillus* and *Clostridium*. These include *B. anthracis*, the causative agent of anthrax, which produces endospores capable of survive for many decades[\[footnote\]](#); *C. tetani* (causes tetanus); *C. difficile* (causes pseudomembranous colitis); *C. perfringens* (causes gas gangrene); and *C. botulinum* (causes botulism). Pathogens such as these are particularly difficult to combat because their endospores are so hard to kill. Special sterilization methods for endospore-forming bacteria are discussed in [Control of Microbial Growth](#).

R. Sinclair et al. “Persistence of Category A Select Agents in the Environment.” *Applied and Environmental Microbiology* 74 no. 3 (2008):555–563.

Note:

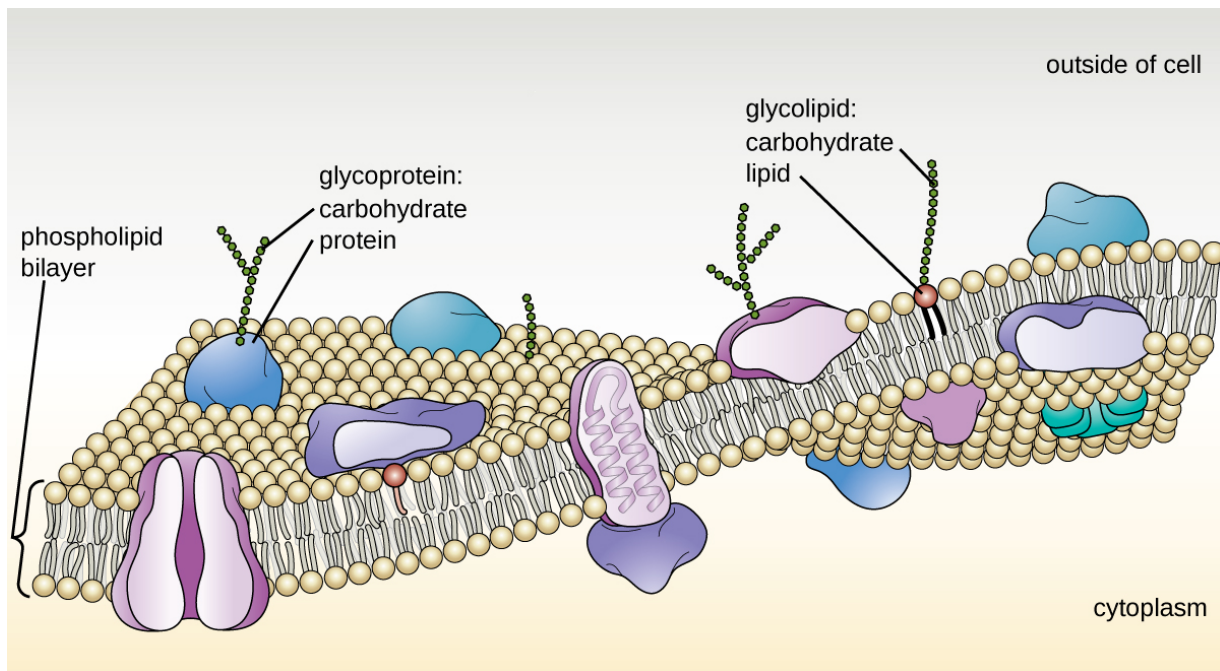
- What is an inclusion?
- What is the function of an endospore?

Plasma Membrane

Structures that enclose the cytoplasm and internal structures of the cell are known collectively as the **cell envelope**. In prokaryotic cells, the structures of the cell envelope vary depending on the type of cell and organism. Most (but not all) prokaryotic cells have a cell wall, but the makeup of this cell wall varies. All cells (prokaryotic and eukaryotic) have a **plasma membrane** (also called **cytoplasmic membrane** or **cell membrane**) that exhibits selective permeability, allowing some molecules to enter or leave the cell while restricting the passage of others.

The structure of the plasma membrane is often described in terms of the **fluid mosaic model**, which refers to the ability of membrane components to move fluidly within the plane of the membrane, as well as the mosaic-like

composition of the components, which include a diverse array of lipid and protein components ([\[link\]](#)). The plasma membrane structure of most bacterial and eukaryotic cell types is a bilayer composed mainly of phospholipids formed with ester linkages and proteins. These phospholipids and proteins have the ability to move laterally within the plane of the membranes as well as between the two phospholipid layers.



The bacterial plasma membrane is a phospholipid bilayer with a variety of embedded proteins that perform various functions for the cell. Note the presence of glycoproteins and glycolipids, whose carbohydrate components extend out from the surface of the cell. The abundance and arrangement of these proteins and lipids can vary greatly between species.

Archaeal membranes are fundamentally different from bacterial and eukaryotic membranes in a few significant ways. First, archaeal membrane phospholipids are formed with ether linkages, in contrast to the ester linkages found in bacterial or eukaryotic cell membranes. Second, archaeal

phospholipids have branched chains, whereas those of bacterial and eukaryotic cells are straight chained. Finally, although some archaeal membranes can be formed of bilayers like those found in bacteria and eukaryotes, other archaeal plasma membranes are lipid monolayers.

Proteins on the cell's surface are important for a variety of functions, including cell-to-cell communication, and sensing environmental conditions and pathogenic virulence factors. Membrane proteins and phospholipids may have carbohydrates (sugars) associated with them and are called glycoproteins or glycolipids, respectively. These glycoprotein and glycolipid complexes extend out from the surface of the cell, allowing the cell to interact with the external environment ([\[link\]](#)). Glycoproteins and glycolipids in the plasma membrane can vary considerably in chemical composition among archaea, bacteria, and eukaryotes, allowing scientists to use them to characterize unique species.

Plasma membranes from different cells types also contain unique phospholipids, which contain fatty acids. As described in [Using Biochemistry to Identify Microorganisms](#), phospholipid-derived fatty acid analysis (PLFA) profiles can be used to identify unique types of cells based on differences in fatty acids. Archaea, bacteria, and eukaryotes each have a unique PFLA profile.

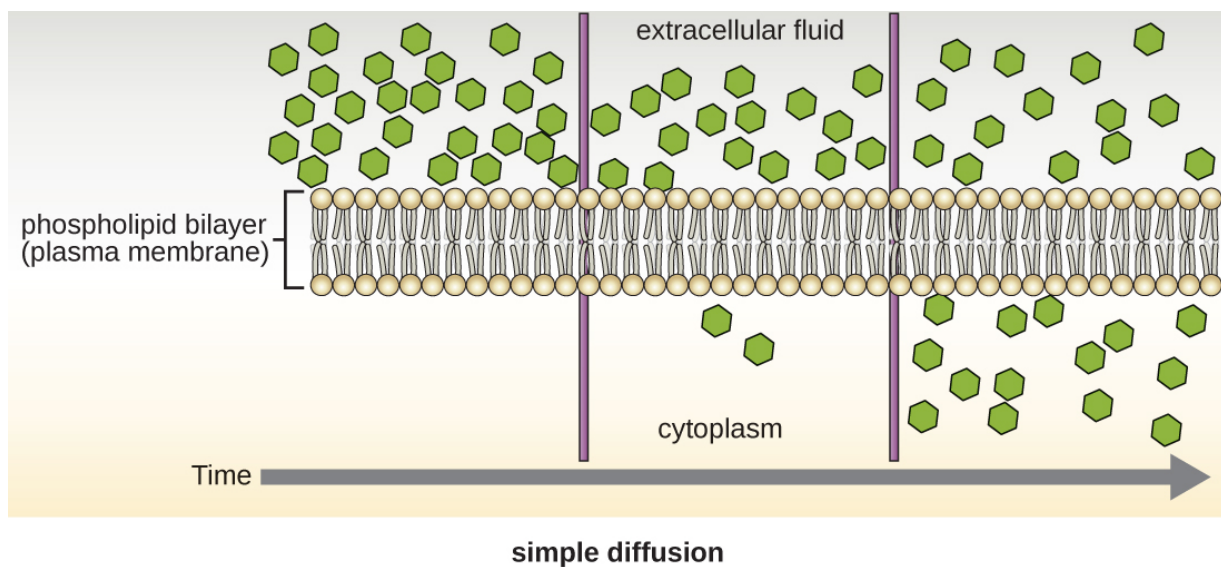
Membrane Transport Mechanisms

One of the most important functions of the plasma membrane is to control the transport of molecules into and out of the cell. Internal conditions must be maintained within a certain range despite any changes in the external environment. The transport of substances across the plasma membrane allows cells to do so.

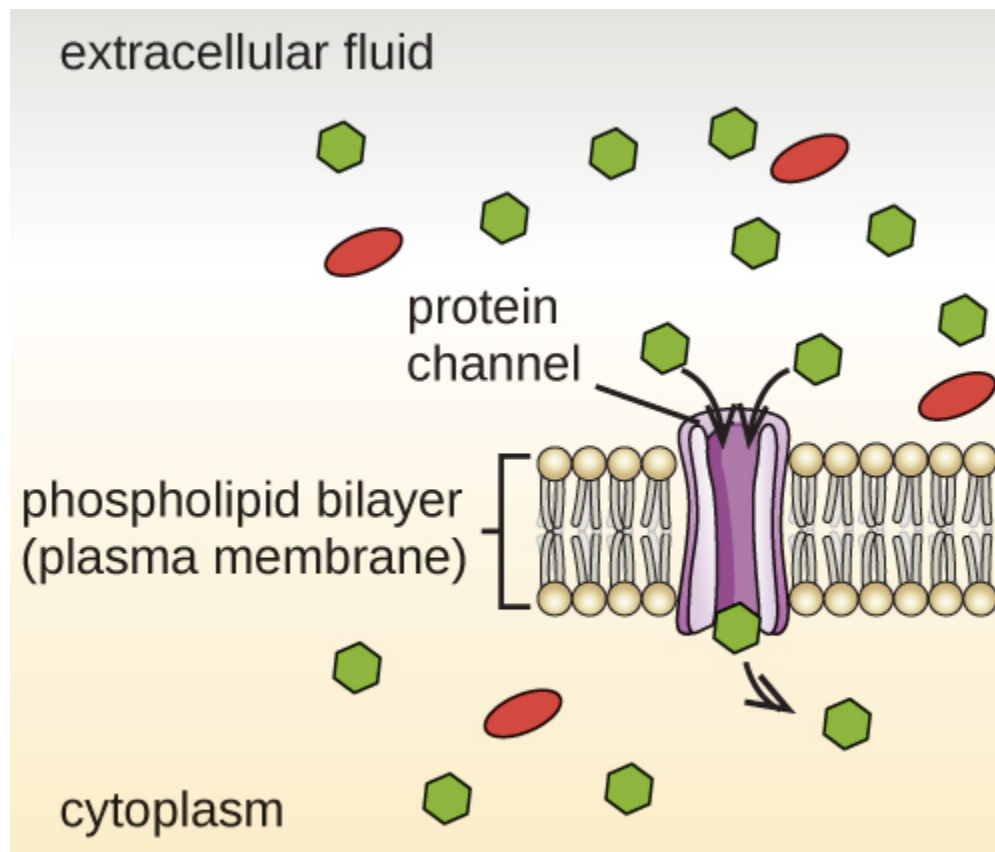
Cells use various modes of transport across the plasma membrane. For example, molecules moving from a higher concentration to a lower concentration with the concentration gradient are transported by simple diffusion, also known as passive transport ([\[link\]](#)). Some small molecules, like carbon dioxide, may cross the membrane bilayer directly by simple

diffusion. However, charged molecules, as well as large molecules, need the help of carriers or channels in the membrane. These structures ferry molecules across the membrane, a process known as facilitated diffusion ([link](#)).

Active transport occurs when cells move molecules across their membrane *against* concentration gradients ([link](#)). A major difference between passive and active transport is that active transport requires adenosine triphosphate (ATP) or other forms of energy to move molecules “uphill.” Therefore, active transport structures are often called “pumps.”

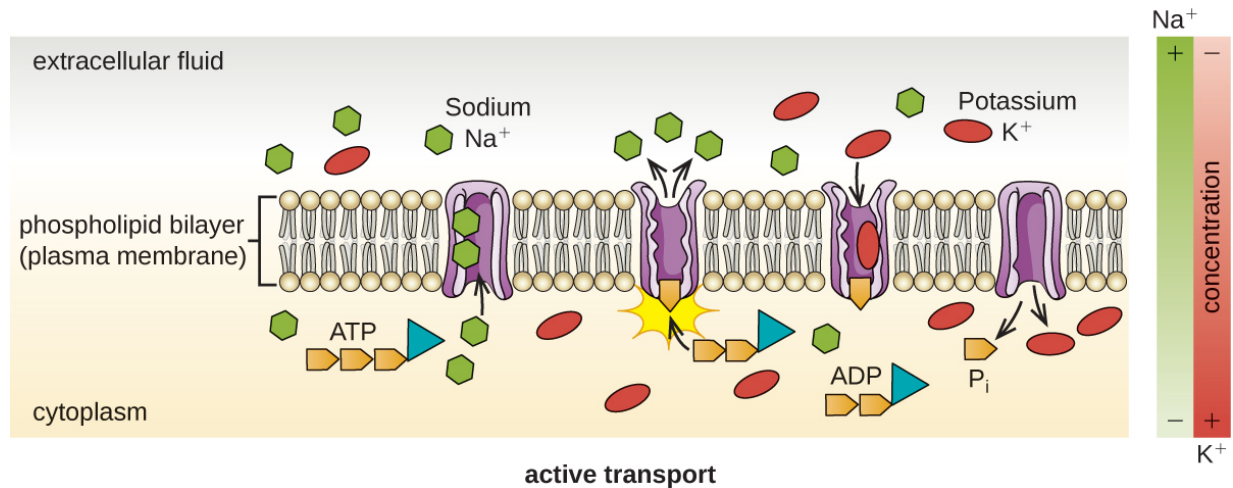


Simple diffusion down a concentration gradient directly across the phospholipid bilayer. (credit: modification of work by Mariana Ruiz Villareal)



facilitated diffusion

Facilitated diffusion down a concentration gradient through a membrane protein. (credit: modification of work by Mariana Ruiz Villareal)



Active transport against a concentration gradient via a membrane pump that requires energy. (credit: modification of work by Mariana Ruiz Villareal)

Group translocation also transports substances into bacterial cells. In this case, as a molecule moves into a cell against its concentration gradient, it is chemically modified so that it does not require transport against an unfavorable concentration gradient. A common example of this is the bacterial phosphotransferase system, a series of carriers that phosphorylates (i.e., adds phosphate ions to) glucose or other sugars upon entry into cells. Since the phosphorylation of sugars is required during the early stages of sugar metabolism, the phosphotransferase system is considered to be an energy neutral system.

Photosynthetic Membrane Structures

Some prokaryotic cells, namely cyanobacteria and photosynthetic bacteria, have membrane structures that enable them to perform photosynthesis. These structures consist of an infolding of the plasma membrane that encloses photosynthetic pigments such as green **chlorophylls** and bacteriochlorophylls. In cyanobacteria, these membrane structures are

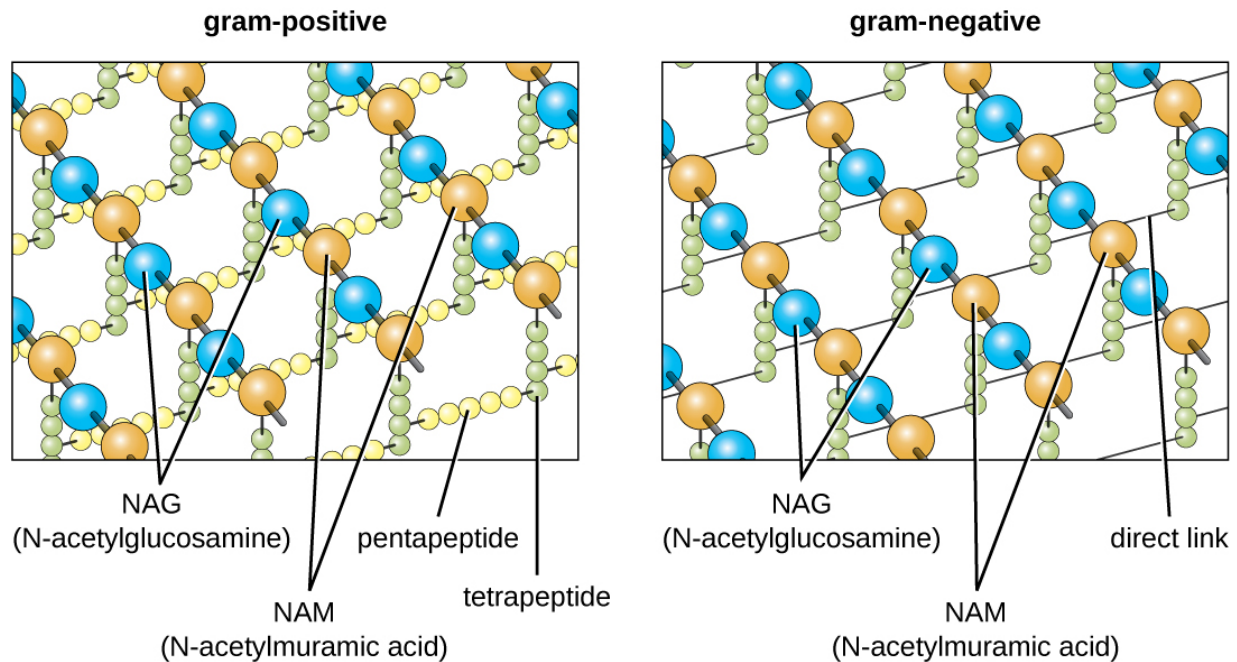
called thylakoids; in photosynthetic bacteria, they are called chromatophores, lamellae, or chlorosomes.

Cell Wall

The primary function of the cell wall is to protect the cell from harsh conditions in the outside environment. When present, there are notable similarities and differences among the cell walls of archaea, bacteria, and eukaryotes.

The major component of bacterial cell walls is called **peptidoglycan** (or murein); it is only found in bacteria. Structurally, peptidoglycan resembles a layer of meshwork or fabric ([\[link\]](#)). Each layer is composed of long chains of alternating molecules of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). The structure of the long chains has significant two-dimensional tensile strength due to the formation of peptide bridges that connect NAG and NAM within each peptidoglycan layer. In gram-negative bacteria, tetrapeptide chains extending from each NAM unit are directly cross-linked, whereas in gram-positive bacteria, these tetrapeptide chains are linked by pentaglycine cross-bridges. Peptidoglycan subunits are made inside of the bacterial cell and then exported and assembled in layers, giving the cell its shape.

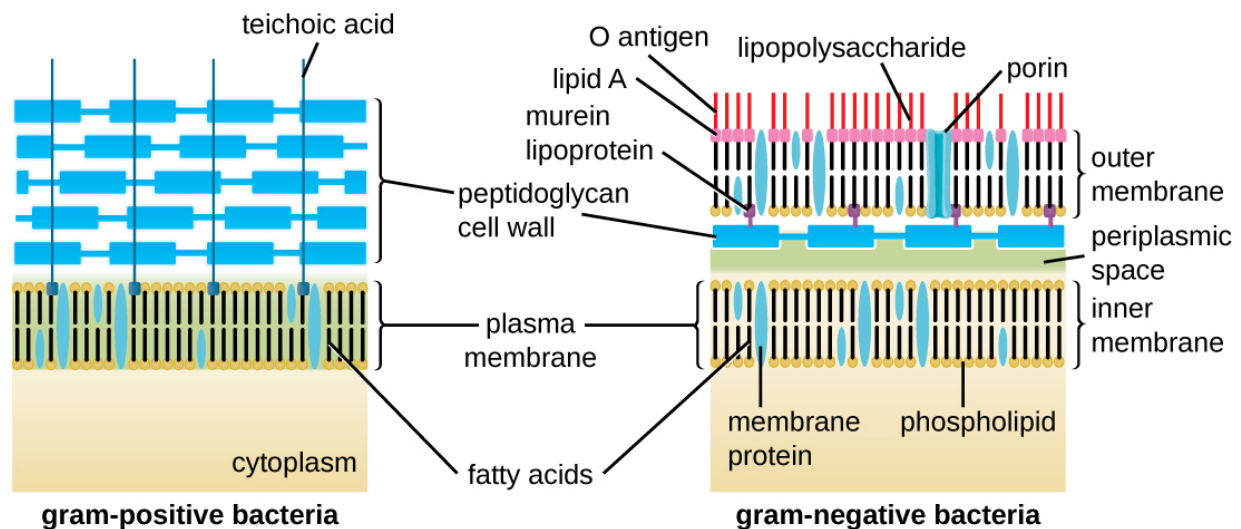
Since peptidoglycan is unique to bacteria, many antibiotic drugs are designed to interfere with peptidoglycan synthesis, weakening the cell wall and making bacterial cells more susceptible to the effects of osmotic pressure (see [Mechanisms of Antibacterial Drugs](#)). In addition, certain cells of the human immune system are able “recognize” bacterial pathogens by detecting peptidoglycan on the surface of a bacterial cell; these cells then engulf and destroy the bacterial cell, using enzymes such as lysozyme, which breaks down and digests the peptidoglycan in their cell walls (see [Pathogen Recognition and Phagocytosis](#)).



Peptidoglycan is composed of polymers of alternating NAM and NAG subunits, which are cross-linked by peptide bridges linking NAM subunits from various glycan chains. This provides the cell wall with tensile strength in two dimensions.

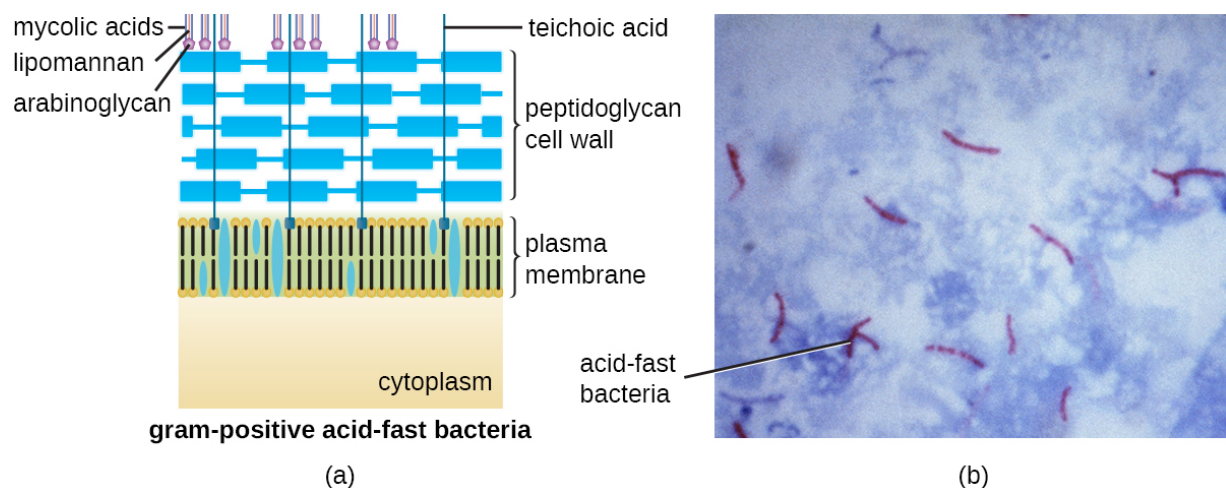
The Gram staining protocol (see [Staining Microscopic Specimens](#)) is used to differentiate two common types of cell wall structures ([\[link\]](#)). Gram-positive cells have a cell wall consisting of many layers of peptidoglycan totaling 30–100 nm in thickness. These peptidoglycan layers are commonly embedded with teichoic acids (TAs), carbohydrate chains that extend through and beyond the peptidoglycan layer.[\[footnote\]](#) TA is thought to stabilize peptidoglycan by increasing its rigidity. TA also plays a role in the ability of pathogenic gram-positive bacteria such as *Streptococcus* to bind to certain proteins on the surface of host cells, enhancing their ability to cause infection. In addition to peptidoglycan and TAs, bacteria of the family Mycobacteriaceae have an external layer of waxy **mycolic acids** in their cell wall; as described in [Staining Microscopic Specimens](#), these bacteria are referred to as acid-fast, since acid-fast stains must be used to penetrate the mycolic acid layer for purposes of microscopy ([\[link\]](#)).

T.J. Silhavy, D. Kahne, S. Walker. "The Bacterial Cell Envelope." *Cold Spring Harbor Perspectives in Biology* 2 no. 5 (2010):a000414.



Bacteria contain two common cell wall structural types. Gram-positive cell walls are structurally simple, containing a thick layer of peptidoglycan with embedded teichoic acid external to the plasma membrane.[\[footnote\]](#) Gram-negative cell walls are structurally more complex, containing three layers: the inner membrane, a thin layer of peptidoglycan, and an outer membrane containing lipopolysaccharide. (credit: modification of work by "Franciscosp2"/Wikimedia Commons)

B. Zuber et al. "Granular Layer in the Periplasmic Space of Gram-Positive Bacteria and Fine Structures of *Enterococcus gallinarum* and *Streptococcus gordonii* Septa Revealed by Cryo-Electron Microscopy of Vitreous Sections." *Journal of Bacteriology* 188 no. 18 (2006):6652–6660

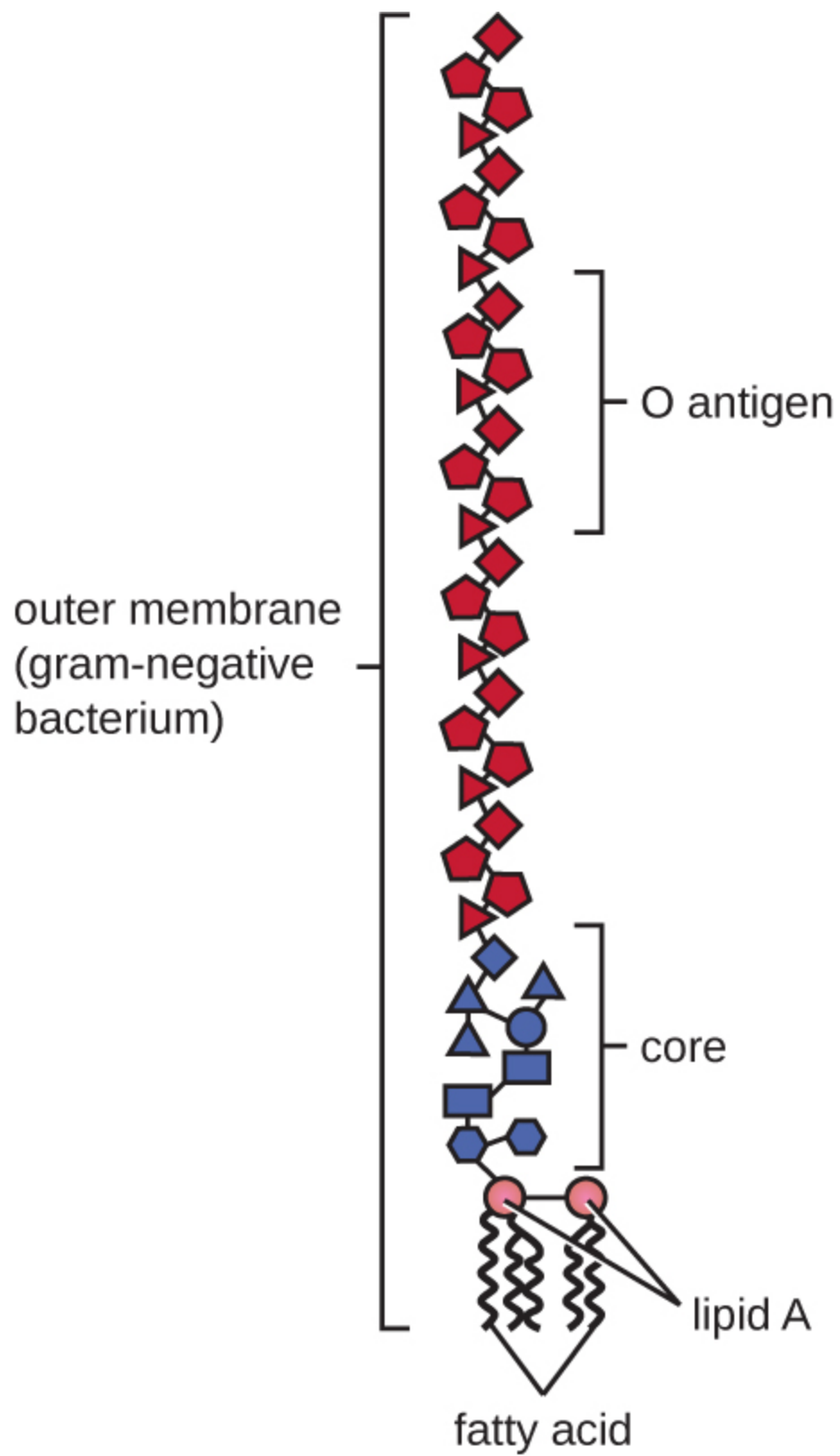


(a) Some gram-positive bacteria, including members of the Mycobacteriaceae, produce waxy mycolic acids found exterior to their structurally-distinct peptidoglycan. (b) The acid-fast staining protocol detects the presence of cell walls that are rich in mycolic acid. Acid-fast cells are stained red by carbolfuchsin. (credit a: modification of work by “Franciscosp2”/Wikimedia Commons; credit b: modification of work by Centers for Disease Control and Prevention)

Gram-negative cells have a much thinner layer of peptidoglycan (no more than about 4 nm thick^{[[footnote](#)]}) than gram-positive cells, and the overall structure of their cell envelope is more complex. In gram-negative cells, a gel-like matrix occupies the **periplasmic space** between the cell wall and the plasma membrane, and there is a second lipid bilayer called the **outer membrane**, which is external to the peptidoglycan layer ([\[link\]](#)). This outer membrane is attached to the peptidoglycan by murein lipoprotein. The outer leaflet of the outer membrane contains the molecule **lipopolysaccharide (LPS)**, which functions as an endotoxin in infections involving gram-negative bacteria, contributing to symptoms such as fever, hemorrhaging, and septic shock. Each LPS molecule is composed of Lipid A, a core polysaccharide, and an O side chain that is composed of sugar-like molecules that comprise the external face of the LPS ([\[link\]](#)). The composition of the O side chain varies between different species and strains of bacteria. Parts of the O side chain called antigens can be detected using serological or immunological tests to identify specific pathogenic strains

like *Escherichia coli* O157:H7, a deadly strain of bacteria that causes bloody diarrhea and kidney failure.

L. Gana, S. Chena, G.J. Jensena. “Molecular Organization of Gram-Negative Peptidoglycan.” *Proceedings of the National Academy of Sciences of the United States of America* 105 no. 48 (2008):18953–18957.



The outer membrane of a gram-negative bacterial

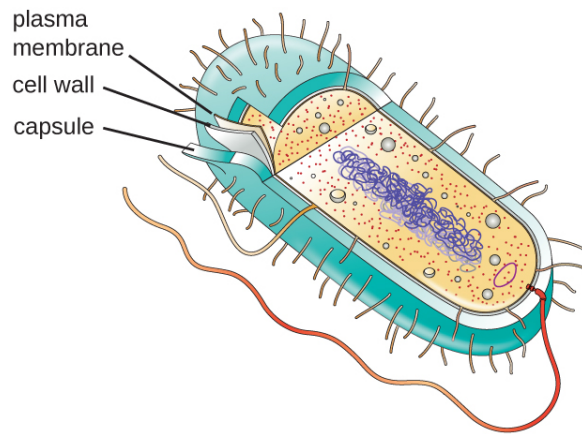
cell contains lipopolysaccharide (LPS), a toxin composed of Lipid A embedded in the outer membrane, a core polysaccharide, and the O side chain.

Archaeal cell wall structure differs from that of bacteria in several significant ways. First, archaeal cell walls do not contain peptidoglycan; instead, they contain a similar polymer called pseudopeptidoglycan (pseudomurein) in which NAM is replaced with a different subunit. Other archaea may have a layer of glycoproteins or polysaccharides that serves as the cell wall instead of pseudopeptidoglycan. Last, as is the case with some bacterial species, there are a few archaea that appear to lack cell walls entirely.

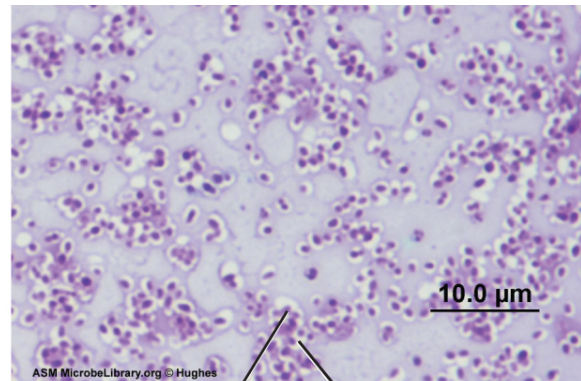
Glycocalyxes and S-Layers

Although most prokaryotic cells have cell walls, some may have additional cell envelope structures exterior to the cell wall, such as glycocalyxes and S-layers. A **glycocalyx** is a sugar coat, of which there are two important types: capsules and slime layers. A **capsule** is an organized layer located outside of the cell wall and usually composed of polysaccharides or proteins ([\[link\]](#)). A **slime layer** is a less tightly organized layer that is only loosely attached to the cell wall and can be more easily washed off. Slime layers may be composed of polysaccharides, glycoproteins, or glycolipids.

Glycocalyxes allows cells to adhere to surfaces, aiding in the formation of biofilms (colonies of microbes that form in layers on surfaces). In nature, most microbes live in mixed communities within biofilms, partly because the biofilm affords them some level of protection. Biofilms generally hold water like a sponge, preventing desiccation. They also protect cells from predation and hinder the action of antibiotics and disinfectants. All of these properties are advantageous to the microbes living in a biofilm, but they present challenges in a clinical setting, where the goal is often to eliminate microbes.



(a)



cell capsule

(b)

(a) Capsules are a type of glycocalyx composed of an organized layer of polysaccharides. (b) A capsule stain of *Pseudomonas aeruginosa*, a bacterial pathogen capable of causing many different types of infections in humans. (credit b: modification of work by American Society for Microbiology)

The ability to produce a capsule can contribute to a microbe's pathogenicity (ability to cause disease) because the capsule can make it more difficult for phagocytic cells (such as white blood cells) to engulf and kill the microorganism. *Streptococcus pneumoniae*, for example, produces a capsule that is well known to aid in this bacterium's pathogenicity. As explained in [Staining Microscopic specimens](#), capsules are difficult to stain for microscopy; negative staining techniques are typically used.

An **S-layer** is another type of cell envelope structure; it is composed of a mixture of structural proteins and glycoproteins. In bacteria, S-layers are found outside the cell wall, but in some archaea, the S-layer serves as the cell wall. The exact function of S-layers is not entirely understood, and they are difficult to study; but available evidence suggests that they may play a variety of functions in different prokaryotic cells, such as helping the cell withstand osmotic pressure and, for certain pathogens, interacting with the host immune system.

Note:**Part 3**

After diagnosing Barbara with pneumonia, the PA writes her a prescription for amoxicillin, a commonly-prescribed type of penicillin derivative. More than a week later, despite taking the full course as directed, Barbara still feels weak and is not fully recovered, although she is still able to get through her daily activities. She returns to the health center for a follow-up visit.

Many types of bacteria, fungi, and viruses can cause pneumonia. Amoxicillin targets the peptidoglycan of bacterial cell walls. Since the amoxicillin has not resolved Barbara's symptoms, the PA concludes that the causative agent probably lacks peptidoglycan, meaning that the pathogen could be a virus, a fungus, or a bacterium that lacks peptidoglycan. Another possibility is that the pathogen is a bacterium containing peptidoglycan but has developed resistance to amoxicillin.

- How can the PA definitively identify the cause of Barbara's pneumonia?
- What form of treatment should the PA prescribe, given that the amoxicillin was ineffective?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

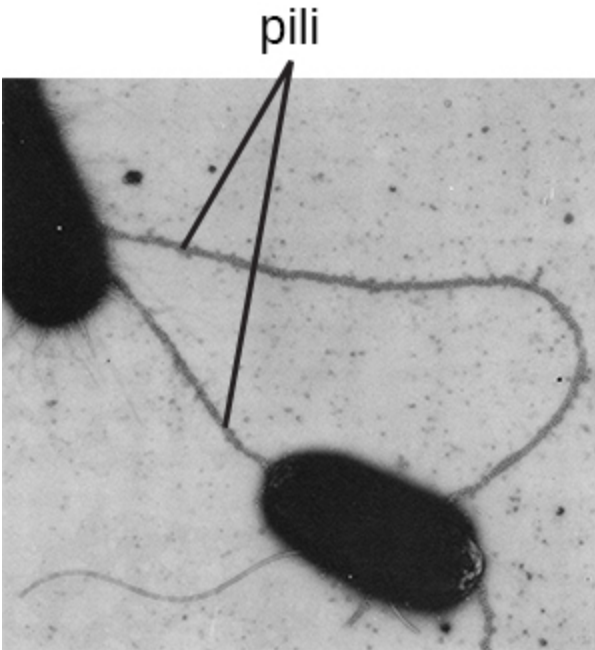
Filamentous Appendages

Many bacterial cells have protein appendages embedded within their cell envelopes that extend outward, allowing interaction with the environment. These appendages can attach to other surfaces, transfer DNA, or provide movement. Filamentous appendages include fimbriae, pili, and flagella.

Fimbriae and Pili

Fimbriae and pili are structurally similar and, because differentiation between the two is problematic, these terms are often used interchangeably. [\[footnote\]](#) [\[footnote\]](#) The term **fimbriae** commonly refers to short bristle-like proteins projecting from the cell surface by the hundreds. Fimbriae enable a cell to attach to surfaces and to other cells. For pathogenic bacteria, adherence to host cells is important for colonization, infectivity, and virulence. Adherence to surfaces is also important in biofilm formation. J.A. Garnetta et al. “Structural Insights Into the Biogenesis and Biofilm Formation by the *Escherichia coli* Common Pilus.” *Proceedings of the National Academy of Sciences of the United States of America* 109 no. 10 (2012):3950–3955. T. Proft, E.N. Baker. “Pili in Gram-Negative and Gram-Positive Bacteria—Structure, Assembly and Their Role in Disease.” *Cellular and Molecular Life Sciences* 66 (2009):613.

The term **pili** (singular: pilus) commonly refers to longer, less numerous protein appendages that aid in attachment to surfaces ([\[link\]](#)). A specific type of pilus, called the **F pilus** or **sex pilus**, is important in the transfer of DNA between bacterial cells, which occurs between members of the same generation when two cells physically transfer or exchange parts of their respective genomes (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)).



Bacteria may produce two different types of protein appendages that aid in surface attachment. Fimbriae typically are more numerous and shorter, whereas pili (shown here) are longer and less numerous per cell. (credit: modification of work by American Society for Microbiology)

Note:**Group A Strep**

Before the structure and function of the various components of the bacterial cell envelope were well understood, scientists were already using cell envelope characteristics to classify bacteria. In 1933, Rebecca Lancefield proposed a method for serotyping various β -hemolytic strains of *Streptococcus* species using an agglutination assay, a technique using

the clumping of bacteria to detect specific cell-surface antigens. In doing so, Lancefield discovered that one group of *S. pyogenes*, found in Group A, was associated with a variety of human diseases. She determined that various strains of Group A strep could be distinguished from each other based on variations in specific cell surface proteins that she named M proteins.

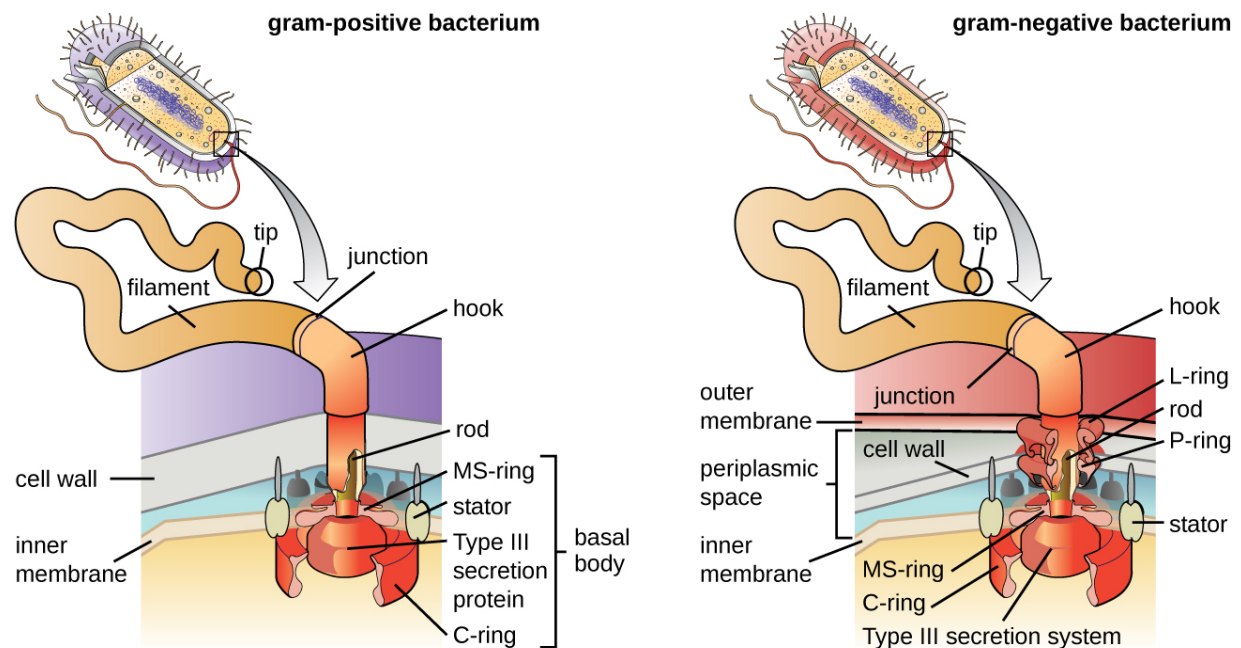
Today, more than 80 different strains of Group A strep have been identified based on M proteins. Various strains of Group A strep are associated with a wide variety of human infections, including streptococcal pharyngitis (strep throat), impetigo, toxic shock syndrome, scarlet fever, rheumatic fever, and necrotizing fasciitis. The M protein is an important virulence factor for Group A strep, helping these strains evade the immune system. Changes in M proteins appear to alter the infectivity of a particular strain of Group A strep.

Flagella

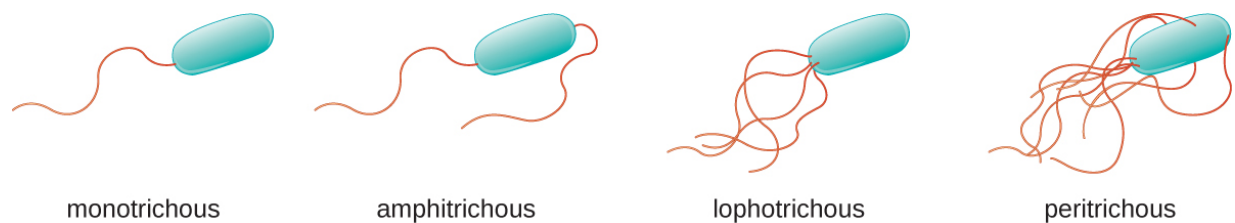
Flagella are structures used by cells to move in aqueous environments. Bacterial flagella act like propellers. They are stiff spiral filaments composed of flagellin protein subunits that extend outward from the cell and spin in solution. The **basal body** is the motor for the flagellum and is embedded in the plasma membrane ([\[link\]](#)). A hook region connects the basal body to the filament. Gram-positive and gram-negative bacteria have different basal body configurations due to differences in cell wall structure.

Different types of motile bacteria exhibit different arrangements of flagella ([\[link\]](#)). A bacterium with a singular flagellum, typically located at one end of the cell (polar), is said to have a **monotrichous** flagellum. An example of a monotrichously flagellated bacterial pathogen is *Vibrio cholerae*, the gram-negative bacterium that causes cholera. Cells with **amphitrichous** flagella have a flagellum or tufts of flagella at each end. An example is *Spirillum minor*, the cause of spirillary (Asian) rat-bite fever or sodoku. Cells with **lophotrichous** flagella have a tuft at one end of the cell. The gram-negative bacillus *Pseudomonas aeruginosa*, an opportunistic pathogen known for causing many infections, including “swimmer’s ear” and burn wound infections, has lophotrichous flagella. Flagella that cover

the entire surface of a bacterial cell are called **peritrichous** flagella. The gram-negative bacterium *E. coli* shows a peritrichous arrangement of flagella.



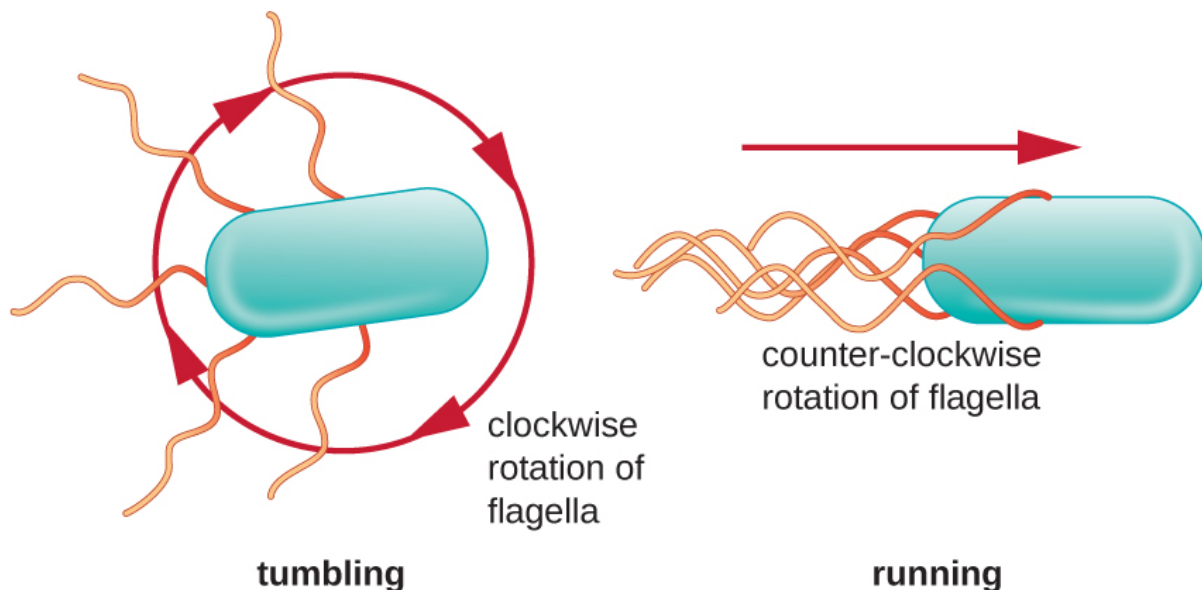
The basic structure of a bacterial flagellum consists of a basal body, hook, and filament. The basal body composition and arrangement differ between gram-positive and gram-negative bacteria. (credit: modification of work by “LadyofHats”/Mariana Ruiz Villareal)



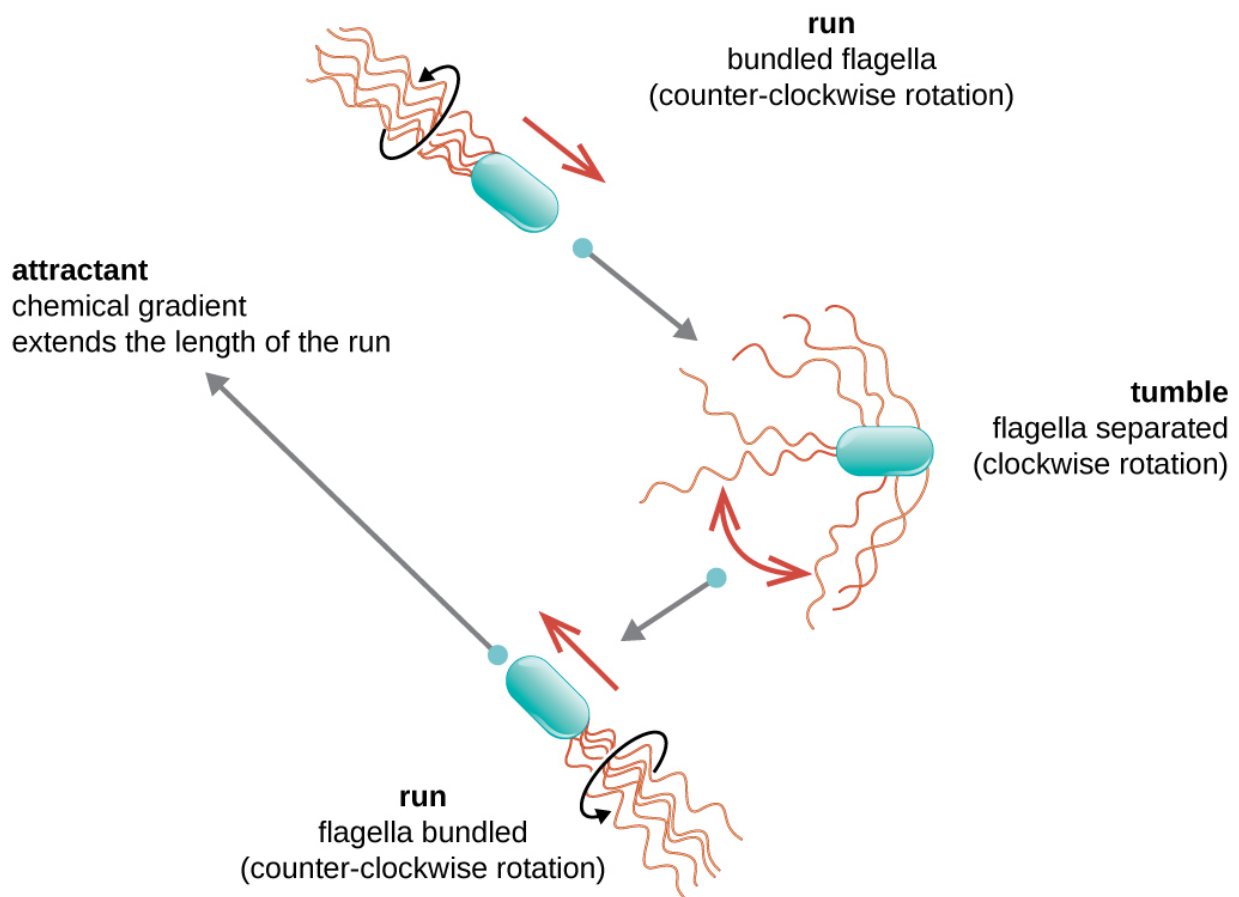
Flagellated bacteria may exhibit multiple arrangements of their flagella. Common arrangements include monotrichous, amphitrichous,

lophotrichous, or peritrichous.

Directional movement depends on the configuration of the flagella. Bacteria can move in response to a variety of environmental signals, including light (**phototaxis**), magnetic fields (**magnetotaxis**) using magnetosomes, and, most commonly, chemical gradients (**chemotaxis**). Purposeful movement toward a chemical attractant, like a food source, or away from a repellent, like a poisonous chemical, is achieved by increasing the length of **runs** and decreasing the length of **tumbles**. When running, flagella rotate in a counterclockwise direction, allowing the bacterial cell to move forward. In a peritrichous bacterium, the flagella are all bundled together in a very streamlined way ([\[link\]](#)), allowing for efficient movement. When tumbling, flagella are splayed out while rotating in a clockwise direction, creating a looping motion and preventing meaningful forward movement but reorienting the cell toward the direction of the attractant. When an attractant exists, runs and tumbles still occur; however, the length of runs is longer, while the length of the tumbles is reduced, allowing overall movement toward the higher concentration of the attractant. When no chemical gradient exists, the lengths of runs and tumbles are more equal, and overall movement is more random ([\[link\]](#)).



Bacteria achieve directional movement by changing the rotation of their flagella. In a cell with peritrichous flagella, the flagella bundle when they rotate in a counterclockwise direction, resulting in a run. However, when the flagella rotate in a clockwise direction, the flagella are no longer bundled, resulting in tumbles.



Without a chemical gradient, flagellar rotation cycles between counterclockwise (run) and clockwise (tumble) with no overall directional movement. However, when a chemical gradient of an attractant exists, the length of runs is extended, while the length of tumbles is decreased. This leads to chemotaxis: an overall directional movement toward the higher concentration of the attractant.

Note:

- What is the peptidoglycan layer and how does it differ between gram-positive and gram-negative bacteria?
- Compare and contrast monotrichous, amphitrichous, lophotrichous, and peritrichous flagella.

Key Concepts and Summary

- Prokaryotic cells differ from eukaryotic cells in that their genetic material is contained in a **nucleoid** rather than a membrane-bound nucleus. In addition, prokaryotic cells generally lack membrane-bound organelles.
- Prokaryotic cells of the same species typically share a similar **cell morphology** and **cellular arrangement**.
- Most prokaryotic cells have a **cell wall** that helps the organism maintain cellular morphology and protects it against changes in osmotic pressure.
- Outside of the nucleoid, prokaryotic cells may contain extrachromosomal DNA in **plasmids**.
- Prokaryotic **ribosomes** that are found in the cytoplasm have a size of 70S.
- Some prokaryotic cells have **inclusions** that store nutrients or chemicals for other uses.
- Some prokaryotic cells are able to form **endospores** through **sporulation** to survive in a dormant state when conditions are unfavorable. Endospores can **germinate**, transforming back into **vegetative cells** when conditions improve.
- In prokaryotic cells, the **cell envelope** includes a **plasma membrane** and usually a cell wall.

- Bacterial membranes are composed of phospholipids with integral or peripheral proteins. The fatty acid components of these phospholipids are ester-linked and are often used to identify specific types of bacteria. The proteins serve a variety of functions, including transport, cell-to-cell communication, and sensing environmental conditions. Archaeal membranes are distinct in that they are composed of fatty acids that are ether-linked to phospholipids.
- Some molecules can move across the bacterial membrane by simple diffusion, but most large molecules must be actively transported through membrane structures using cellular energy.
- Prokaryotic cell walls may be composed of **peptidoglycan** (bacteria) or **pseudopeptidoglycan** (archaea).
- Gram-positive bacterial cells are characterized by a thick **peptidoglycan** layer, whereas gram-negative bacterial cells are characterized by a thin peptidoglycan layer surrounded by an outer membrane.
- Some prokaryotic cells produce **glycocalyx** coatings, such as **capsules** and **slime layers**, that aid in attachment to surfaces and/or evasion of the host immune system.
- Some prokaryotic cells have **fimbriae** or **pili**, filamentous appendages that aid in attachment to surfaces. Pili are also used in the transfer of genetic material between cells.
- Some prokaryotic cells use one or more **flagella** to move through water. **Peritrichous** bacteria, which have numerous flagella, use **runs** and **tumbles** to move purposefully in the direction of a chemical attractant.

Multiple Choice

Exercise:

Problem:

Which of the following terms refers to a prokaryotic cell that is comma shaped?

- A. coccus
- B. coccobacilli

- C. vibrio
 - D. spirillum
-

Solution:

C

Exercise:

Problem:

Which bacterial structures are important for adherence to surfaces?
(Select all that apply.)

- A. endospores
 - B. cell walls
 - C. fimbriae
 - D. capsules
 - E. flagella
-

Solution:

C, D

Exercise:

Problem:

Which of the following cell wall components is unique to gram-negative cells?

- A. lipopolysaccharide
 - B. teichoic acid
 - C. mycolic acid
 - D. peptidoglycan
-

Solution:

A

Exercise:

Problem:

Which of the following terms refers to a bacterial cell having a single tuft of flagella at one end?

- A. monotrichous
- B. amphitrichous
- C. peritrichous
- D. lophotrichous

Solution:

D

Exercise:

Problem:

Bacterial cell walls are primarily composed of which of the following?

- A. phospholipid
- B. protein
- C. carbohydrate
- D. peptidoglycan

Solution:

D

True/False

Exercise:

Problem:

Bacteria have 80S ribosomes each composed of a 60S large subunit and a 40S small subunit.

Solution:

False

Fill in the Blank**Exercise:****Problem:**

Prokaryotic cells that are rod-shaped are called _____.

Solution:

bacilli

Exercise:**Problem:**

The type of inclusion containing polymerized inorganic phosphate is called _____.

Solution:

volutin (or metachromatic granule)

Short Answer**Exercise:**

Problem:

What is the direction of water flow for a bacterial cell living in a hypotonic environment? How do cell walls help bacteria living in such environments?

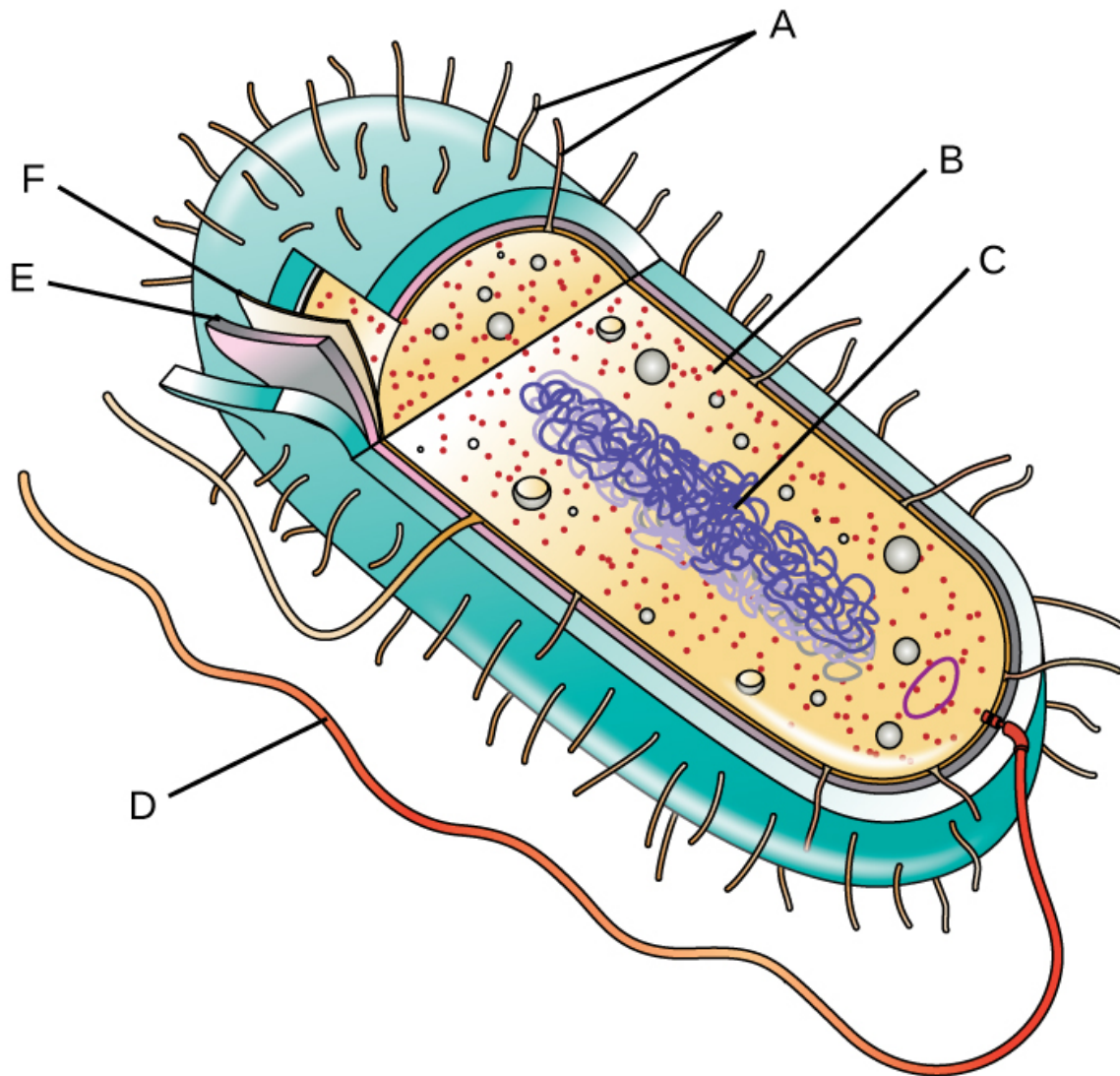
Exercise:

Problem:

How do bacterial flagella respond to a chemical gradient of an attractant to move toward a higher concentration of the chemical?

Exercise:

Problem: Label the parts of the prokaryotic cell.



Critical Thinking

Exercise:

Problem:

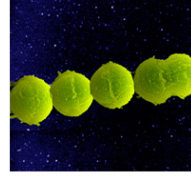
Which of the following slides is a good example of staphylococci?



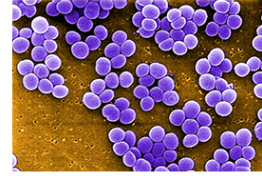
A



B



C



D

(credit a: modification of work by U.S. Department of Agriculture; credit b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by NIAID)

Exercise:

Problem:

Provide some examples of bacterial structures that might be used as antibiotic targets and explain why.

Exercise:

Problem:

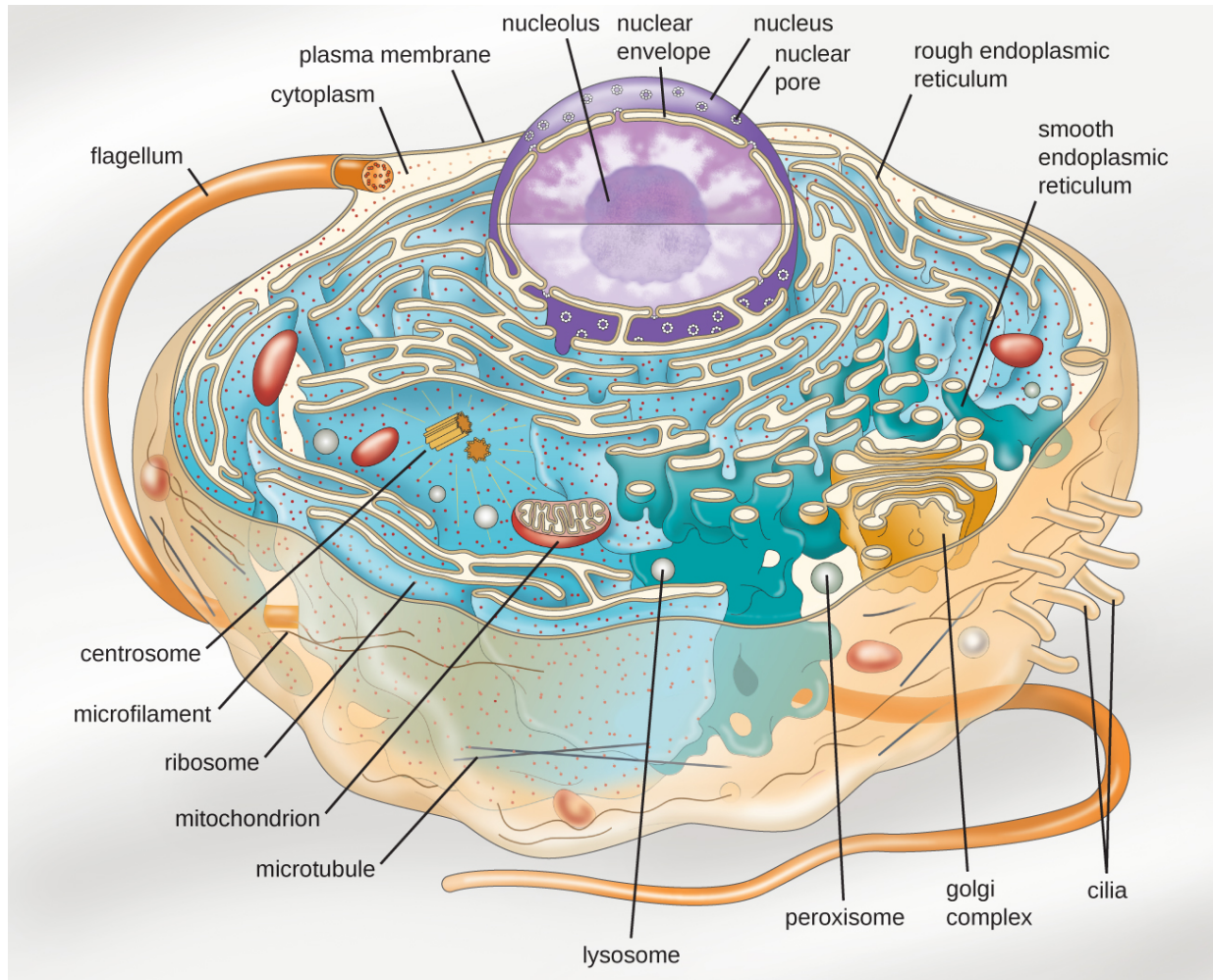
The causative agent of botulism, a deadly form of food poisoning, is an endospore-forming bacterium called *Clostridium botulinum*. Why might it be difficult to kill this bacterium in contaminated food?

Unique Characteristics of Eukaryotic Cells

LEARNING OBJECTIVES

- Explain the distinguishing characteristics of eukaryotic cells
- Describe internal and external structures of eukaryotic cells in terms of their physical structure, chemical structure, and function
- Identify and describe structures and organelles unique to eukaryotic cells
- Compare and contrast similar structures found in prokaryotic and eukaryotic cells

Eukaryotic organisms include protozoans, algae, fungi, plants, and animals. Some eukaryotic cells are independent, single-celled microorganisms, whereas others are part of multicellular organisms. The cells of eukaryotic organisms have several distinguishing characteristics. Above all, eukaryotic cells are defined by the presence of a nucleus surrounded by a complex nuclear membrane. Also, eukaryotic cells are characterized by the presence of membrane-bound organelles in the cytoplasm. Organelles such as mitochondria, the endoplasmic reticulum (ER), Golgi apparatus, lysosomes, and peroxisomes are held in place by the **cytoskeleton**, an internal network that supports transport of intracellular components and helps maintain cell shape ([\[link\]](#)). The genome of eukaryotic cells is packaged in multiple, rod-shaped chromosomes as opposed to the single, circular-shaped chromosome that characterizes most prokaryotic cells. [\[link\]](#) compares the characteristics of eukaryotic cell structures with those of bacteria and archaea.



An illustration of a generalized, single-celled eukaryotic organism. Note that cells of eukaryotic organisms vary greatly in terms of structure and function, and a particular cell may not have all of the structures shown here.

Summary of Cell Structures			
Cell Structure	Prokaryotes		Eukaryotes
	Bacteria	Archaea	
Size	~0.5–1 μM	~0.5–1 μM	~5–20 μM

Summary of Cell Structures			
Cell Structure	Prokaryotes		Eukaryotes
	Bacteria	Archaea	
Surface area-to-volume ratio	High	High	Low
Nucleus	No	No	Yes
Genome characteristics	• Single chromosome • Circular • Haploid • Lacks histones	• Single chromosome • Circular • Haploid • Contains histones	• Multiple chromosomes • Linear • Haploid or diploid • Contains histones
Cell division	Binary fission	Binary fission	Mitosis, meiosis
Membrane lipid composition	• Ester-linked • Straight-chain fatty acids • Bilayer	• Ether-linked • Branched isoprenoids • Bilayer or monolayer	• Ester-linked • Straight-chain fatty acids • Sterols • Bilayer
Cell wall composition	• Peptidoglycan, or • None	• Pseudopeptidoglycan, or • Glycopeptide, or • Polysaccharide, or • Protein (S-layer), or • None	• Cellulose (plants, some algae) • Chitin (molluscs, insects, crustaceans, and fungi) • Silica (some algae) • Most others lack cell walls

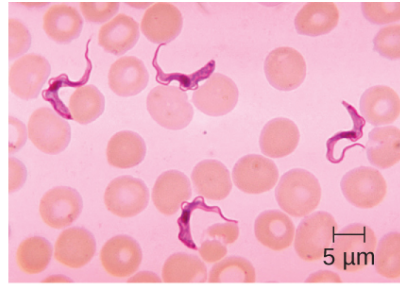
Summary of Cell Structures			
Cell Structure	Prokaryotes		Eukaryotes
	Bacteria	Archaea	
Motility structures	Rigid spiral flagella composed of flagellin	Rigid spiral flagella composed of archaeal flagellins	Flexible flagella and cilia composed of microtubules
Membrane-bound organelles	No	No	Yes
Endomembrane system	No	No	Yes (ER, Golgi, lysosomes)
Ribosomes	70S	70S	• 80S in cytoplasm and rough ER • 70S in mitochondria, chloroplasts

Cell Morphologies

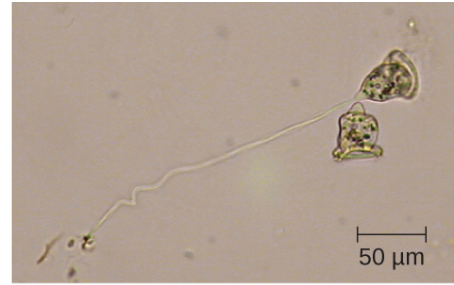
Eukaryotic cells display a wide variety of different cell morphologies. Possible shapes include spheroid, ovoid, cuboidal, cylindrical, flat, lenticular, fusiform, discoidal, crescent, ring stellate, and polygonal ([link](#)). Some eukaryotic cells are irregular in shape, and some are capable of changing shape. The shape of a particular type of eukaryotic cell may be influenced by factors such as its primary function, the organization of its cytoskeleton, the viscosity of its cytoplasm, the rigidity of its cell membrane or cell wall (if it has one), and the physical pressure exerted on it by the surrounding environment and/or adjoining cells.



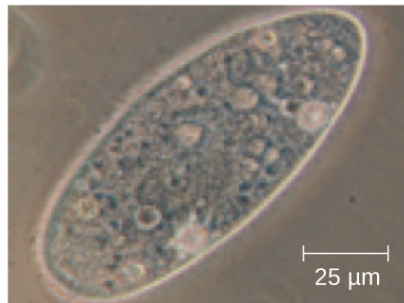
(a)



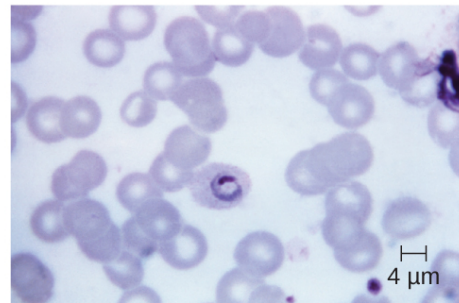
(b)



(c)



(d)



(e)

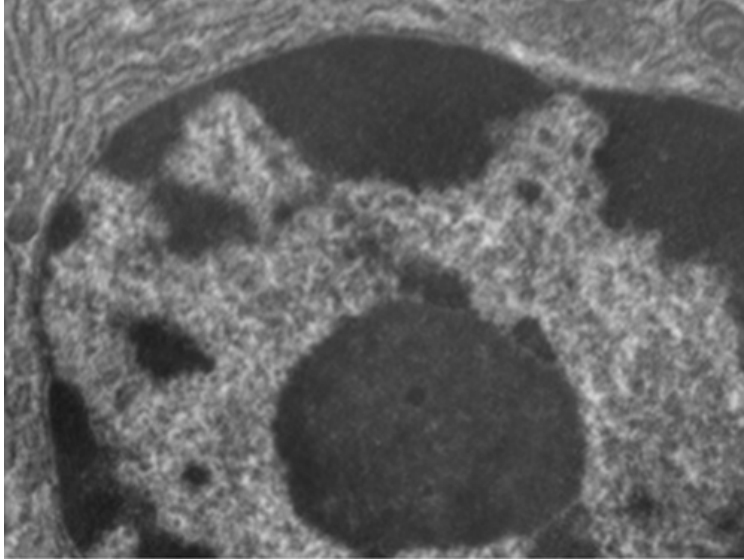
Eukaryotic cells come in a variety of cell shapes. (a) Spheroid *Chromulina* alga. (b) Fusiform shaped *Trypanosoma*. (c) Bell-shaped *Vorticella*. (d) Ovoid *Paramecium*. (e) Ring-shaped *Plasmodium ovale*. (credit a: modification of work by NOAA; credit b, e: modification of work by Centers for Disease Control and Prevention)

Note:

- Identify two differences between eukaryotic and prokaryotic cells.

Nucleus

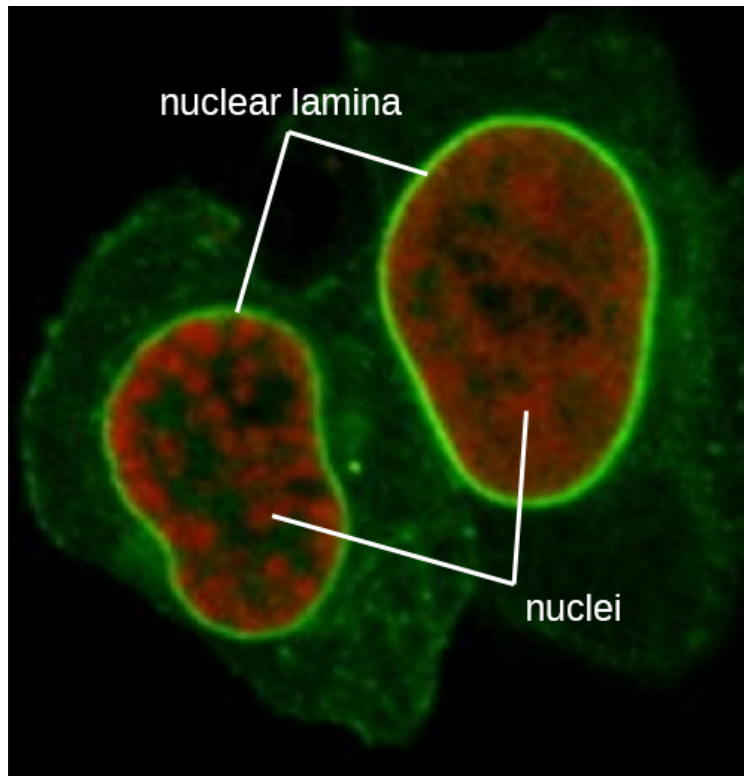
Unlike prokaryotic cells, in which DNA is loosely contained in the nucleoid region, eukaryotic cells possess a **nucleus**, which is surrounded by a complex nuclear membrane that houses the DNA genome ([\[link\]](#)). By containing the cell's DNA, the nucleus ultimately controls all activities of the cell and also serves an essential role in reproduction and heredity. Eukaryotic cells typically have their DNA organized into multiple linear chromosomes. The DNA within the nucleus is highly organized and condensed to fit inside the nucleus, which is accomplished by wrapping the DNA around proteins called histones.



Eukaryotic cells have a well-defined nucleus surrounded by a nuclear membrane. The nucleus of this mammalian lung cell is located in the bottom right corner of the image. The large, dark, oval-shaped structure within the nucleus is the nucleolus.

Although most eukaryotic cells have only one nucleus, exceptions exist. For example, protozoans of the genus *Paramecium* typically have two complete nuclei: a small nucleus that is used for reproduction (micronucleus) and a large nucleus that directs cellular metabolism (macronucleus). Additionally, some fungi transiently form cells with two nuclei, called heterokaryotic cells, during sexual reproduction. Cells whose nuclei divide, but whose cytoplasm does not, are called **coenocytes**.

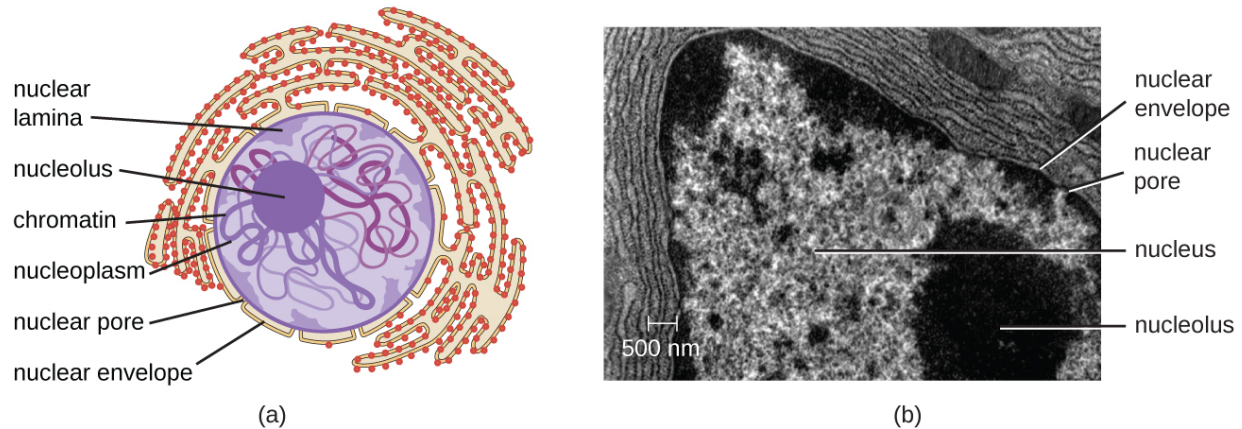
The nucleus is bound by a complex **nuclear membrane**, often called the **nuclear envelope**, that consists of two distinct lipid bilayers that are contiguous with each other ([link](#)). Despite these connections between the inner and outer membranes, each membrane contains unique lipids and proteins on its inner and outer surfaces. The nuclear envelope contains nuclear pores, which are large, rosette-shaped protein complexes that control the movement of materials into and out of the nucleus. The overall shape of the nucleus is determined by the **nuclear lamina**, a meshwork of intermediate filaments found just inside the nuclear envelope membranes. Outside the nucleus, additional intermediate filaments form a looser mesh and serve to anchor the nucleus in position within the cell.



In this fluorescent microscope image, all the intermediate filaments have been stained with a bright green fluorescent stain. The nuclear lamina is the intense bright green ring around the faint red nuclei.

Nucleolus

The **nucleolus** is a dense region within the nucleus where ribosomal RNA (rRNA) biosynthesis occurs. In addition, the nucleolus is also the site where assembly of ribosomes begins. Preribosomal complexes are assembled from rRNA and proteins in the nucleolus; they are then transported out to the cytoplasm, where ribosome assembly is completed ([link](#)).



(a) The nucleolus is the dark, dense area within the nucleus. It is the site of rRNA synthesis and preribosomal assembly. (b) Electron micrograph showing the nucleolus.

Ribosomes

Ribosomes found in eukaryotic organelles such as mitochondria or chloroplasts have 70S ribosomes—the same size as prokaryotic ribosomes. However, nonorganelle-associated ribosomes in eukaryotic cells are **80S ribosomes**, composed of a 40S small subunit and a 60S large subunit. In terms of size and composition, this makes them distinct from the ribosomes of prokaryotic cells.

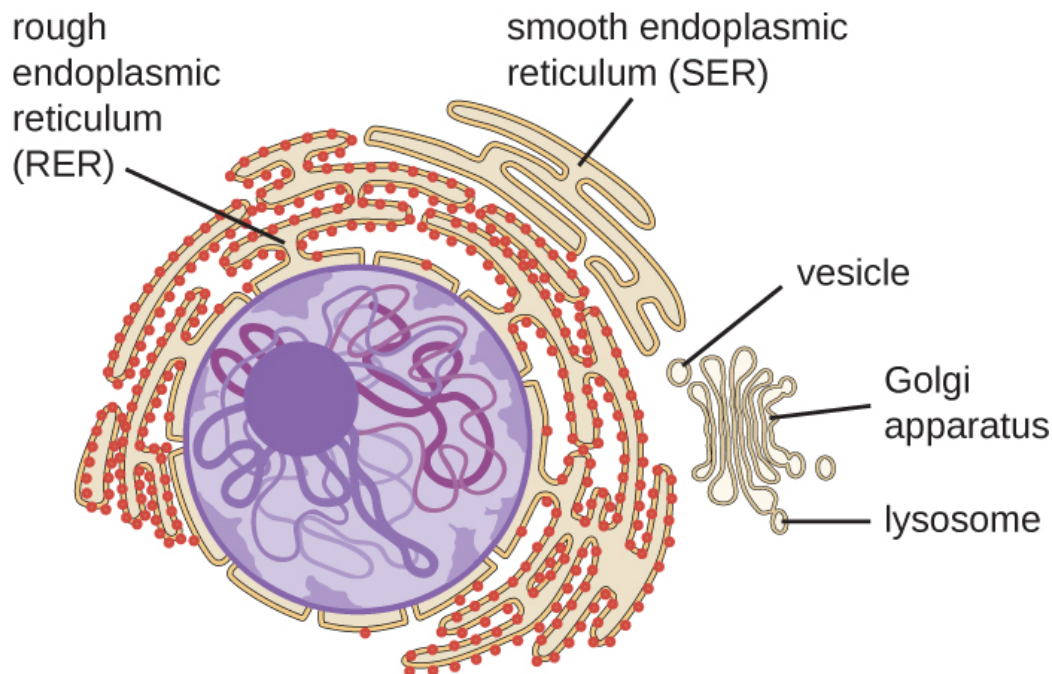
The two types of nonorganelle-associated eukaryotic ribosomes are defined by their location in the cell: **free ribosomes** and **membrane-bound ribosomes**. Free ribosomes are found in the cytoplasm and serve to synthesize water-soluble proteins; membrane-bound ribosomes are found attached to the rough endoplasmic reticulum and make proteins for insertion into the cell membrane or proteins destined for export from the cell.

The differences between eukaryotic and prokaryotic ribosomes are clinically relevant because certain antibiotic drugs are designed to target one or the other. For example, cycloheximide targets eukaryotic action, whereas chloramphenicol targets prokaryotic ribosomes.^[footnote] Since human cells are eukaryotic, they generally are not harmed by antibiotics that destroy the prokaryotic ribosomes in bacteria. However, sometimes negative side effects may occur because mitochondria in human cells contain prokaryotic ribosomes.

A.E. Barnhill, M.T. Brewer, S.A. Carlson. “Adverse Effects of Antimicrobials via Predictable or Idiosyncratic Inhibition of Host Mitochondrial Components.” *Antimicrobial Agents and Chemotherapy* 56 no. 8 (2012):4046–4051.

Endomembrane System

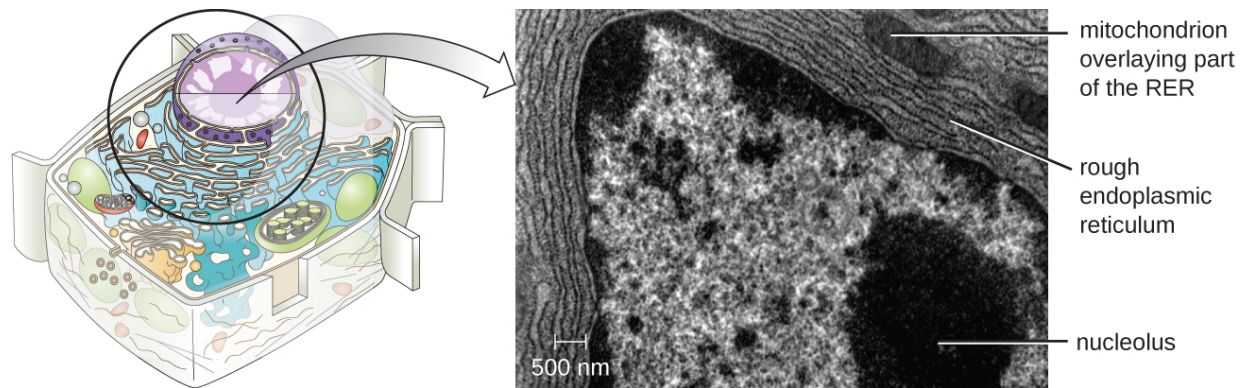
The **endomembrane system**, unique to eukaryotic cells, is a series of membranous tubules, sacs, and flattened disks that synthesize many cell components and move materials around within the cell ([link](#)). Because of their larger cell size, eukaryotic cells require this system to transport materials that cannot be dispersed by diffusion alone. The endomembrane system comprises several organelles and connections between them, including the endoplasmic reticulum, Golgi apparatus, lysosomes, and vesicles.



The endomembrane system is composed of a series of membranous intracellular structures that facilitate movement of materials throughout the cell and to the cell membrane.

Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** is an interconnected array of tubules and **cisternae** (flattened sacs) with a single lipid bilayer ([link](#)). The spaces inside of the cisternae are called **lumen** of the ER. There are two types of ER, **rough endoplasmic reticulum (RER)** and **smooth endoplasmic reticulum (SER)**. These two different types of ER are sites for the synthesis of distinctly different types of molecules. RER is studded with ribosomes bound on the cytoplasmic side of the membrane. These ribosomes make proteins destined for the plasma membrane ([link](#)). Following synthesis, these proteins are inserted into the membrane of the RER. Small sacs of the RER containing these newly synthesized proteins then bud off as **transport vesicles** and move either to the Golgi apparatus for further processing, directly to the plasma membrane, to the membrane of another organelle, or out of the cell. Transport vesicles are single-lipid, bilayer, membranous spheres with hollow interiors that carry molecules. SER does not have ribosomes and, therefore, appears “smooth.” It is involved in biosynthesis of lipids, carbohydrate metabolism, and detoxification of toxic compounds within the cell.



The rough endoplasmic reticulum is studded with ribosomes for the synthesis of membrane proteins (which give it its rough appearance).

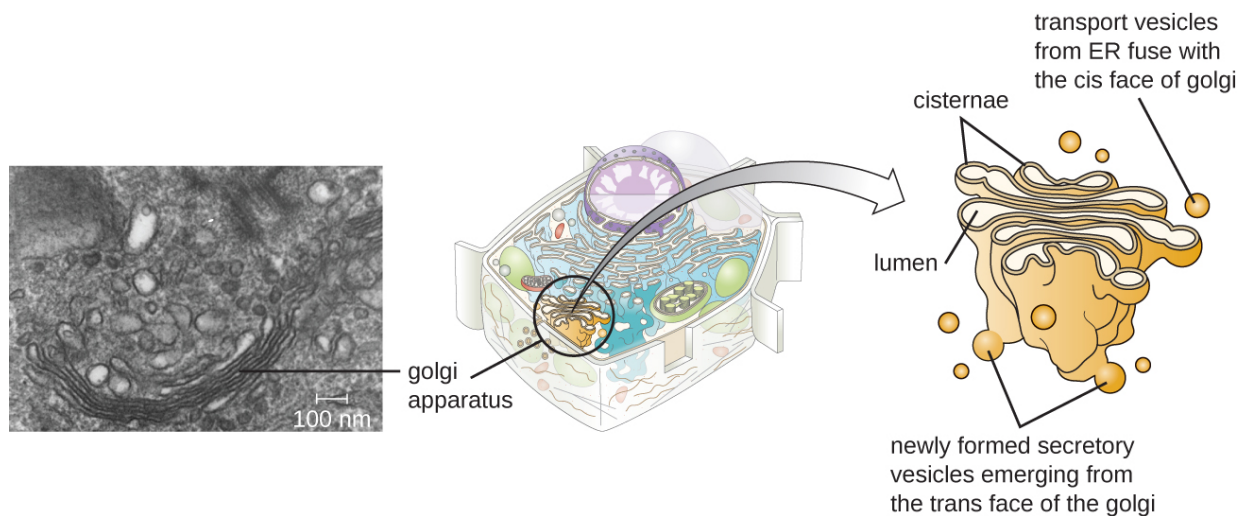
Golgi Apparatus

The Golgi apparatus was discovered within the endomembrane system in 1898 by Italian scientist Camillo Golgi (1843–1926), who developed a novel staining technique that showed stacked membrane structures within the cells of *Plasmodium*, the causative agent of malaria. The **Golgi apparatus** is composed of a series of membranous disks called dictyosomes, each having a single lipid bilayer, that are stacked together ([link](#)).

Enzymes in the Golgi apparatus modify lipids and proteins transported from the ER to the Golgi, often adding carbohydrate components to them, producing glycolipids, glycoproteins, or proteoglycans. Glycolipids and glycoproteins are often inserted into the plasma membrane and are important for signal recognition by other cells or infectious particles. Different types of cells can be distinguished from one another by the structure and arrangement of the glycolipids and glycoproteins contained in their plasma membranes. These glycolipids and glycoproteins commonly also serve as cell surface receptors.

Transport vesicles leaving the ER fuse with a Golgi apparatus on its receiving, or *cis*, face. The proteins are processed within the Golgi apparatus, and then additional transport vesicles containing the modified proteins and lipids pinch off from the Golgi apparatus on its outgoing, or *trans*, face. These outgoing vesicles move to and fuse with the plasma membrane or the membrane of other organelles.

Exocytosis is the process by which **secretory vesicles** (spherical membranous sacs) release their contents to the cell's exterior ([link](#)). All cells have constitutive secretory pathways in which secretory vesicles transport soluble proteins that are released from the cell continually (constitutively). Certain specialized cells also have regulated secretory pathways, which are used to store soluble proteins in secretory vesicles. Regulated secretion involves substances that are only released in response to certain events or signals. For example, certain cells of the human immune system (e.g., mast cells) secrete histamine in response to the presence of foreign objects or pathogens in the body. Histamine is a compound that triggers various mechanisms used by the immune system to eliminate pathogens.



A transmission electron micrograph (left) of a Golgi apparatus in a white blood cell. The illustration (right) shows the cup-shaped, stacked disks and several transport vesicles. The Golgi apparatus modifies lipids and proteins, producing glycolipids and glycoproteins, respectively, which are commonly inserted into the plasma membrane.

Lysosomes

In the 1960s, Belgian scientist Christian de Duve (1917–2013) discovered **lysosomes**, membrane-bound organelles of the endomembrane system that contain digestive enzymes. Certain types of eukaryotic cells use lysosomes to break down various particles, such as food, damaged organelles or cellular debris, microorganisms, or immune complexes. Compartmentalization of the digestive enzymes within the lysosome allows the cell to efficiently digest matter without harming the cytoplasmic components of the cell.

Note:

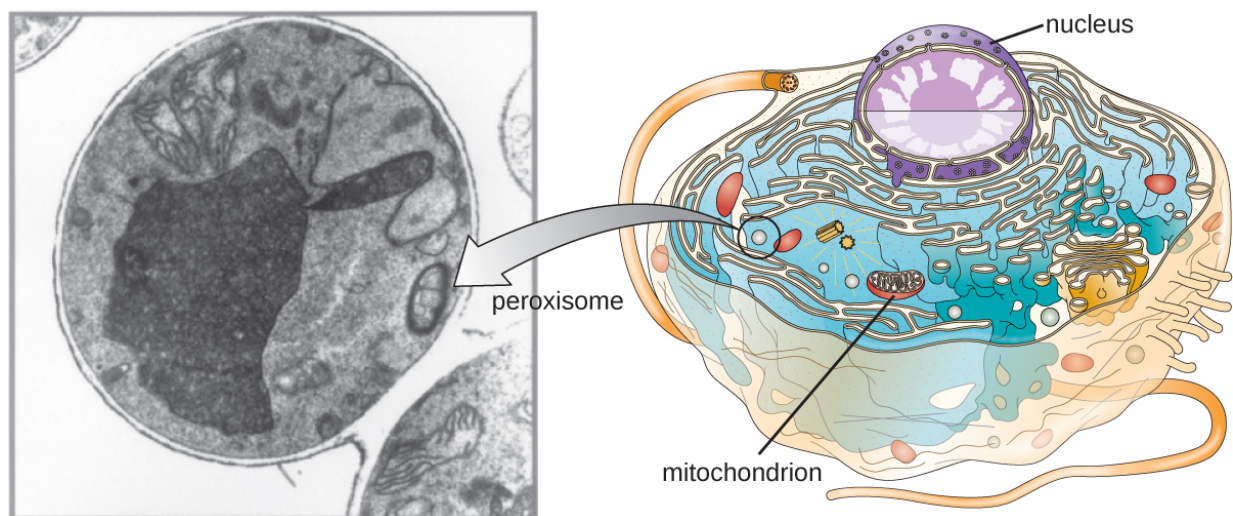
- Name the components of the endomembrane system and describe the function of each component.

Peroxisomes

Christian de Duve is also credited with the discovery of **peroxisomes**, membrane-bound organelles that are not part of the endomembrane system ([link](#)). Peroxisomes form independently in the cytoplasm from the synthesis of peroxin proteins by free ribosomes and the incorporation of these peroxin proteins into existing peroxisomes. Growing peroxisomes then divide by a process similar to binary fission.

Peroxisomes were first named for their ability to produce hydrogen peroxide, a highly reactive molecule that helps to break down molecules such as uric acid, amino acids, and fatty acids. Peroxisomes also possess the enzyme catalase, which can degrade hydrogen peroxide. Along with the SER, peroxisomes also play a role in lipid biosynthesis. Like lysosomes, the compartmentalization of these degradative molecules within an organelle helps protect the cytoplasmic contents from unwanted damage.

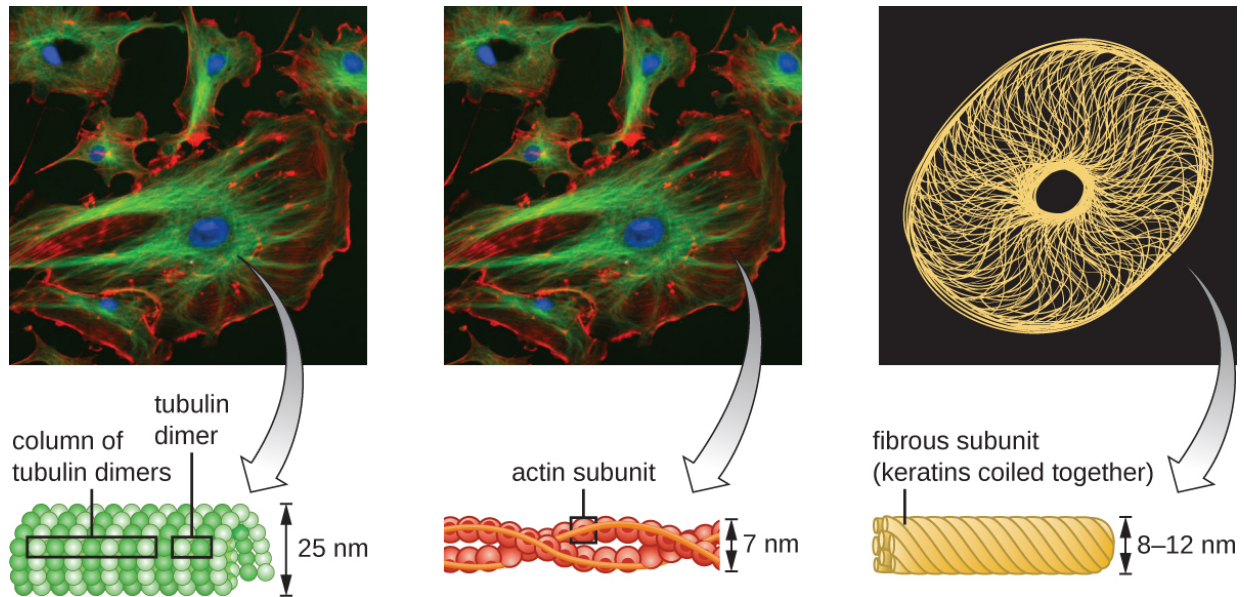
The peroxisomes of certain organisms are specialized to meet their particular functional needs. For example, glyoxysomes are modified peroxisomes of yeasts and plant cells that perform several metabolic functions, including the production of sugar molecules. Similarly, glycosomes are modified peroxisomes made by certain trypanosomes, the pathogenic protozoans that cause Chagas disease and African sleeping sickness.



A transmission electron micrograph (left) of a cell containing a peroxisome. The illustration (right) shows the location of peroxisomes in a cell. These eukaryotic structures play a role in lipid biosynthesis and breaking down various molecules. They may also have other specialized functions depending on the cell type. (credit “micrograph”: modification of work by American Society for Microbiology)

Cytoskeleton

Eukaryotic cells have an internal cytoskeleton made of **microfilaments**, **intermediate filaments**, and **microtubules**. This matrix of fibers and tubes provides structural support as well as a network over which materials can be transported within the cell and on which organelles can be anchored ([link](#)). For example, the process of exocytosis involves the movement of a vesicle via the cytoskeletal network to the plasma membrane, where it can release its contents.

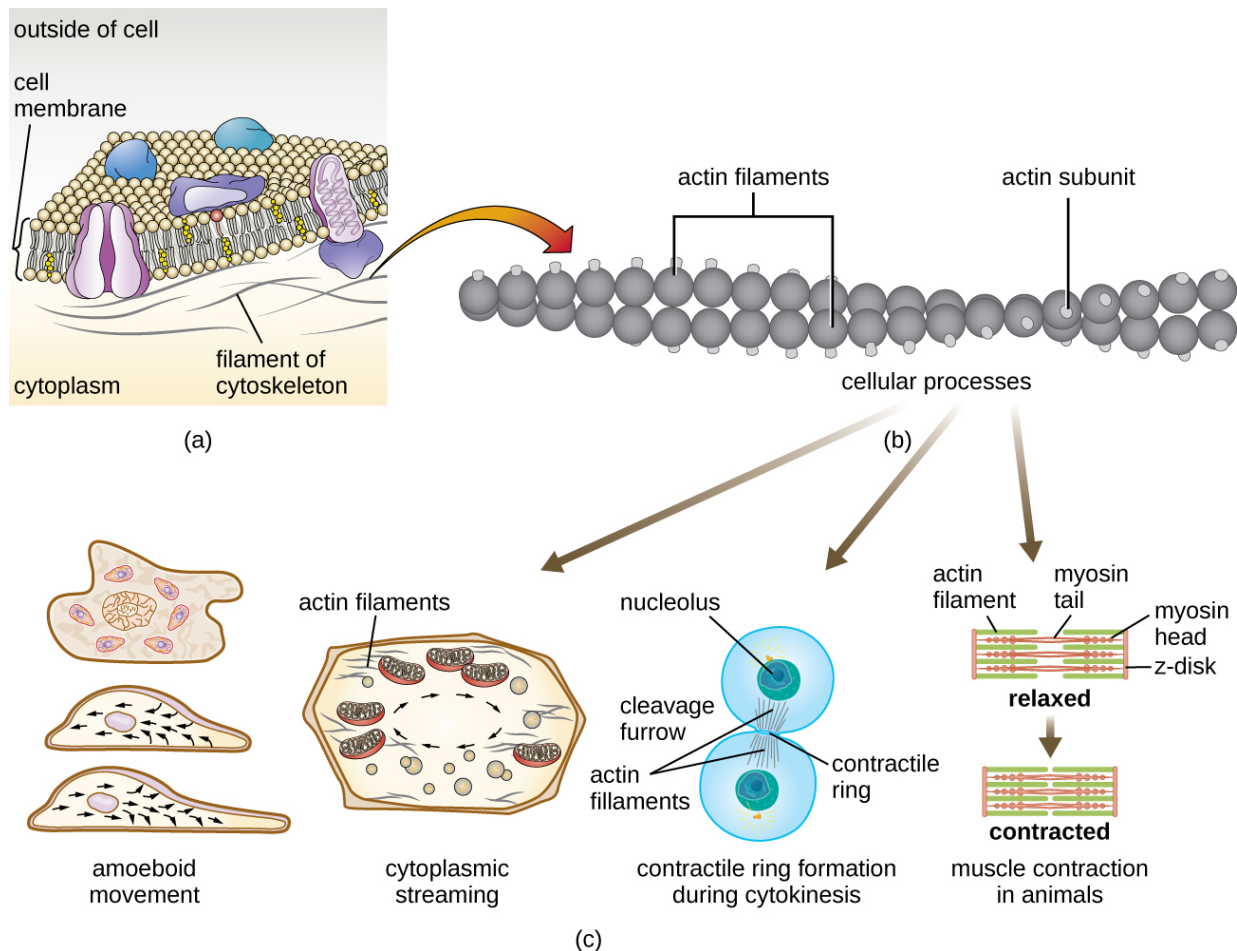


The cytoskeleton is a network of microfilaments, intermediate filaments, and microtubules found throughout the cytoplasm of a eukaryotic cell. In these fluorescently labeled animal cells, the microtubules are green, the actin microfilaments are red, the nucleus is blue, and keratin (a type of intermediate filament) is yellow.

Microfilaments are composed of two intertwined strands of actin, each composed of **actin** monomers forming filamentous cables 6 nm in diameter^[footnote] ([link](#)). The actin filaments work together with motor proteins, like myosin, to effect muscle contraction in animals or the amoeboid movement of some eukaryotic microbes. In amoeboid organisms, actin can be found in two forms: a stiffer, polymerized, gel form and a more fluid, unpolymerized soluble form. Actin in the gel form creates stability in the ectoplasm, the gel-like area of cytoplasm just inside the plasma membrane of amoeboid protozoans.

Fuchs E, Cleveland DW. "A Structural Scaffolding of Intermediate Filaments in Health and Disease." *Science* 279 no. 5350 (1998):514–519.

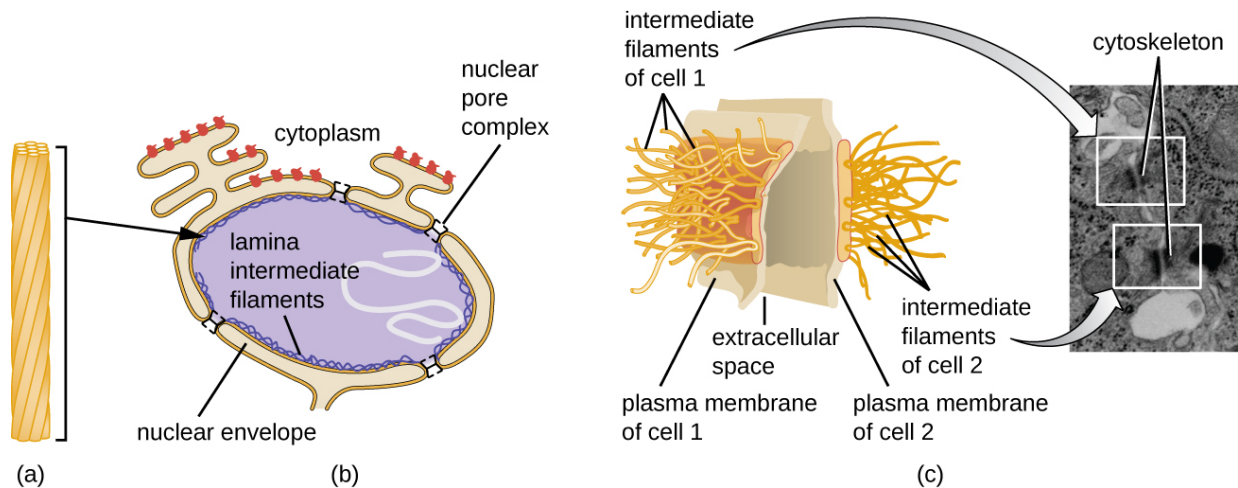
Temporary extensions of the cytoplasmic membrane called **pseudopodia** (meaning "false feet") are produced through the forward flow of soluble actin filaments into the pseudopodia, followed by the gel-sol cycling of the actin filaments, resulting in cell motility. Once the cytoplasm extends outward, forming a pseudopodium, the remaining cytoplasm flows up to join the leading edge, thereby creating forward locomotion. Beyond amoeboid movement, microfilaments are also involved in a variety of other processes in eukaryotic cells, including cytoplasmic streaming (the movement or circulation of cytoplasm within the cell), cleavage furrow formation during cell division, and muscle movement in animals ([link](#)). These functions are the result of the dynamic nature of microfilaments, which can polymerize and depolymerize relatively easily in response to cellular signals, and their interactions with molecular motors in different types of eukaryotic cells.



(a) A microfilament is composed of a pair of actin filaments. (b) Each actin filament is a string of polymerized actin monomers. (c) The dynamic nature of actin, due to its polymerization and depolymerization and its association with myosin, allows microfilaments to be involved in a variety of cellular processes, including amoeboid movement, cytoplasmic streaming, contractile ring formation during cell division, and muscle contraction in animals.

Intermediate filaments ([link](#)) are a diverse group of cytoskeletal filaments that act as cables within the cell. They are termed “intermediate” because their 10-nm diameter is thicker than that of actin but thinner than that of microtubules.[footnote](#) They are composed of several strands of polymerized subunits that, in turn, are made up of a wide variety of monomers. Intermediate filaments tend to be more permanent in the cell and maintain the position of the nucleus. They also form the nuclear lamina (lining or layer) just inside the nuclear envelope. Additionally, intermediate filaments play a role in anchoring cells together in animal tissues. The intermediate filament protein desmin is found in desmosomes, the protein structures that join muscle cells together and help them resist external physical forces. The intermediate filament protein keratin is a structural protein found in hair, skin, and nails.

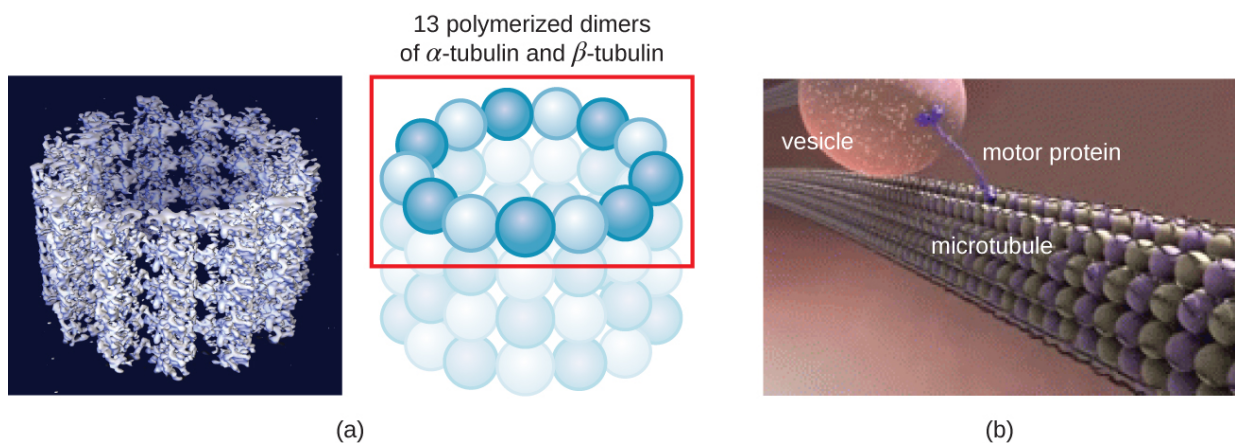
E. Fuchs, D.W. Cleveland. “A Structural Scaffolding of Intermediate Filaments in Health and Disease.” *Science* 279 no. 5350 (1998):514–519.



(a) Intermediate filaments are composed of multiple strands of polymerized subunits. They are more permanent than other cytoskeletal structures and serve a variety of functions. (b) Intermediate filaments form much of the nuclear lamina. (c) Intermediate filaments form the desmosomes between cells in some animal tissues. (credit c “illustration”: modification of work by Mariana Ruiz Villareal)

Microtubules ([\[link\]](#)) are a third type of cytoskeletal fiber composed of tubulin dimers (α tubulin and β tubulin). These form hollow tubes 23 nm in diameter that are used as girders within the cytoskeleton. [\[footnote\]](#) Like microfilaments, microtubules are dynamic and have the ability to rapidly assemble and disassemble. Microtubules also work with motor proteins (such as dynein and kinesin) to move organelles and vesicles around within the cytoplasm. Additionally, microtubules are the main components of eukaryotic flagella and cilia, composing both the filament and the basal body components ([\[link\]](#)).

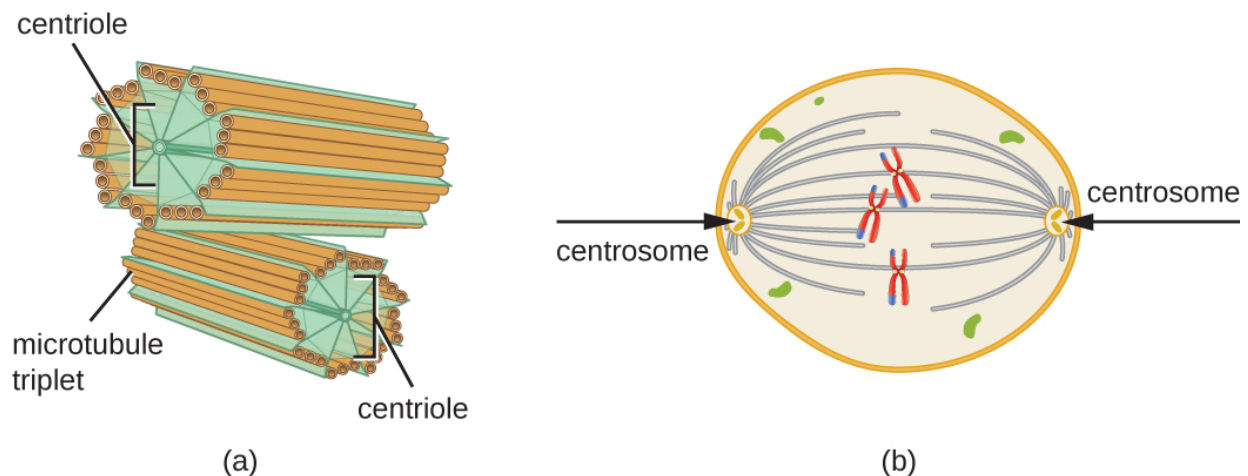
E. Fuchs, D.W. Cleveland. “A Structural Scaffolding of Intermediate Filaments in Health and Disease.” *Science* 279 no. 5350 (1998):514–519.



(a) Microtubules are hollow structures composed of polymerized tubulin dimers. (b) They are involved in several cellular processes, including the movement of organelles throughout the

cytoplasm. Motor proteins carry organelles along microtubule tracks that crisscross the entire cell. (credit b: modification of work by National Institute on Aging)

In addition, microtubules are involved in cell division, forming the mitotic spindle that serves to separate chromosomes during mitosis and meiosis. The mitotic spindle is produced by two **centrosomes**, which are essentially microtubule-organizing centers, at opposite ends of the cell. Each centrosome is composed of a pair of **centrioles** positioned at right angles to each other, and each centriole is an array of nine parallel microtubules arranged in triplets ([link](#)).



(a) A centrosome is composed of two centrioles positioned at right angles to each other. Each centriole is composed of nine triplets of microtubules held together by accessory proteins. (b) In animal cells, the centrosomes (arrows) serve as microtubule-organizing centers of the mitotic spindle during mitosis.

Note:

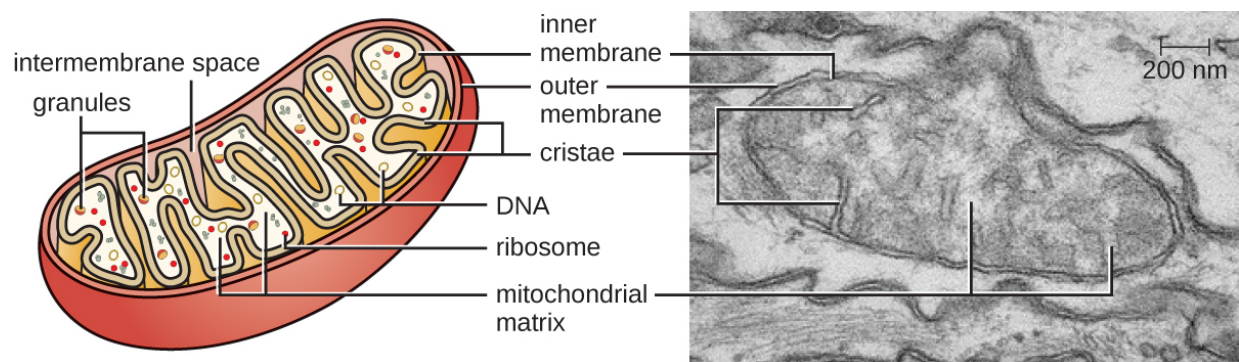
- Compare and contrast the three types of cytoskeletal structures described in this section.

Mitochondria

The large, complex organelles in which aerobic cellular respiration occurs in eukaryotic cells are called **mitochondria** ([link](#)). The term “mitochondrion” was first coined by German microbiologist Carl Benda in 1898 and was later connected with the process of respiration by Otto Warburg in 1913. Scientists during the 1960s discovered that mitochondria have their own genome and 70S ribosomes. The mitochondrial genome was found to be bacterial, when it was sequenced in 1976. These findings

ultimately supported the endosymbiotic theory proposed by Lynn Margulis, which states that mitochondria originally arose through an endosymbiotic event in which a bacterium capable of aerobic cellular respiration was taken up by phagocytosis into a host cell and remained as a viable intracellular component.

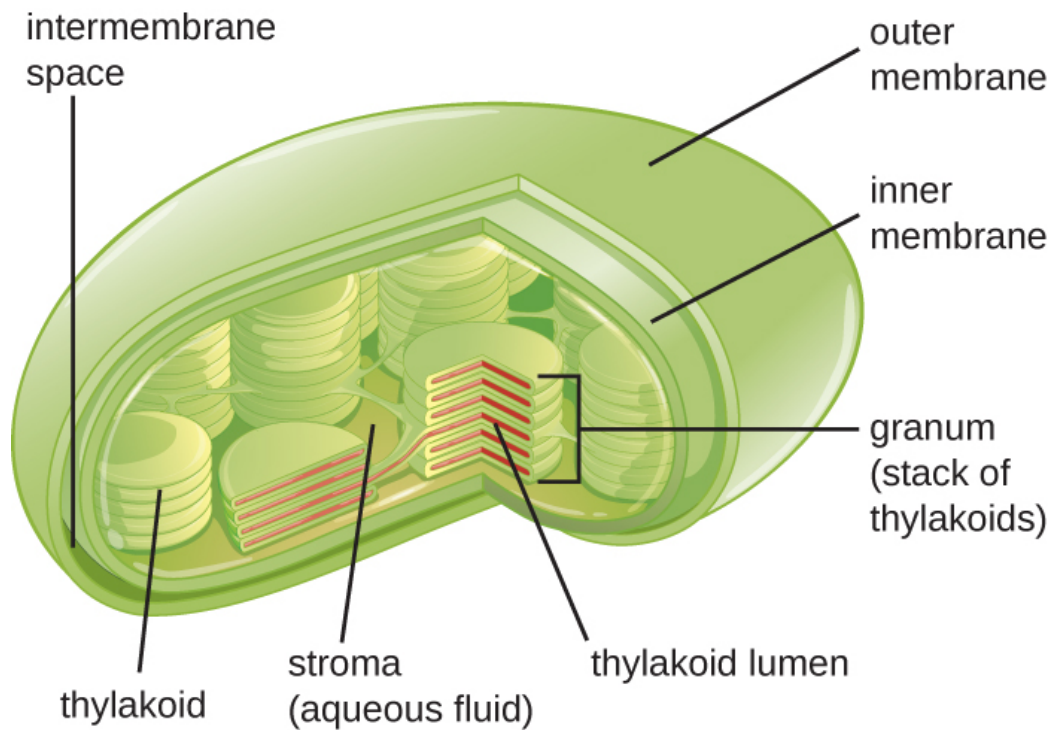
Each mitochondrion has two lipid membranes. The outer membrane is a remnant of the original host cell's membrane structures. The inner membrane was derived from the bacterial plasma membrane. The electron transport chain for aerobic respiration uses integral proteins embedded in the inner membrane. The **mitochondrial matrix**, corresponding to the location of the original bacterium's cytoplasm, is the current location of many metabolic enzymes. It also contains mitochondrial DNA and 70S ribosomes. Invaginations of the inner membrane, called cristae, evolved to increase surface area for the location of biochemical reactions. The folding patterns of the cristae differ among various types of eukaryotic cells and are used to distinguish different eukaryotic organisms from each other.



Each mitochondrion is surrounded by two membranes, the inner of which is extensively folded into cristae and is the site of the intermembrane space. The mitochondrial matrix contains the mitochondrial DNA, ribosomes, and metabolic enzymes. The transmission electron micrograph of a mitochondrion, on the right, shows both membranes, including cristae and the mitochondrial matrix. (credit “micrograph”: modification of work by Matthew Britton; scale-bar data from Matt Russell)

Chloroplasts

Plant cells and algal cells contain **chloroplasts**, the organelles in which photosynthesis occurs ([link](#)). All chloroplasts have at least three membrane systems: the outer membrane, the inner membrane, and the thylakoid membrane system. Inside the outer and inner membranes is the chloroplast **stroma**, a gel-like fluid that makes up much of a chloroplast's volume, and in which the **thylakoid** system floats. The thylakoid system is a highly dynamic collection of folded membrane sacs. It is where the green photosynthetic pigment chlorophyll is found and the light reactions of photosynthesis occur. In most plant chloroplasts, the thylakoids are arranged in stacks called grana (singular: granum), whereas in some algal chloroplasts, the thylakoids are free floating.



Photosynthesis takes place in chloroplasts, which have an outer membrane and an inner membrane. Stacks of thylakoids called grana form a third membrane layer.

Other organelles similar to mitochondria have arisen in other types of eukaryotes, but their roles differ. Hydrogenosomes are found in some anaerobic eukaryotes and serve as the location of anaerobic hydrogen production. Hydrogenosomes typically lack their own DNA and ribosomes. Kinetoplasts are a variation of the mitochondria found in some eukaryotic pathogens. In these organisms, each cell has a single, long, branched mitochondrion in which kinetoplast DNA, organized as multiple circular pieces of DNA, is found concentrated at one pole of the cell.

Note:

Mitochondria-Related Organelles in Protozoan Parasites

Many protozoans, including several protozoan parasites that cause infections in humans, can be identified by their unusual appearance. Distinguishing features may include complex cell morphologies, the presence of unique organelles, or the absence of common organelles. The protozoan parasites *Giardia lamblia* and *Trichomonas vaginalis* are two examples.

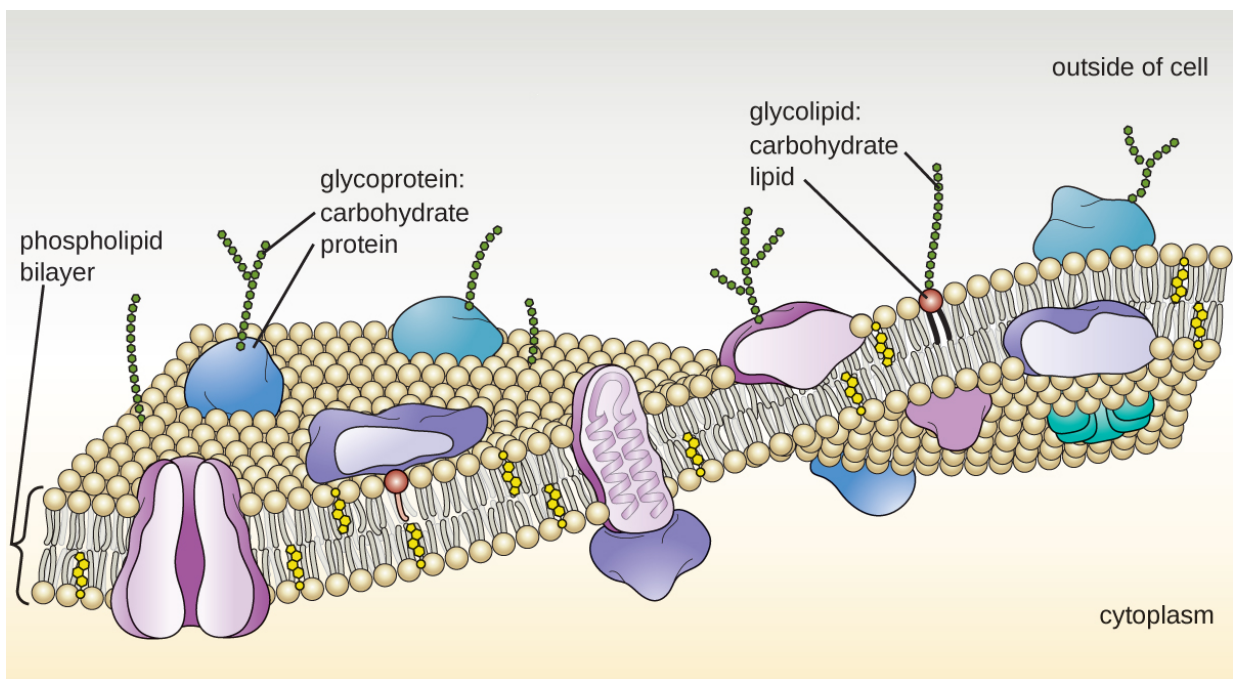
G. lamblia, a frequent cause of diarrhea in humans and many other animals, is an anaerobic parasite that possesses two nuclei and several flagella. Its Golgi apparatus and endoplasmic reticulum are greatly reduced, and it lacks mitochondria completely. However, it does have organelles known as mitosomes, double-membrane-bound organelles that appear to be severely reduced mitochondria. This has led scientists to believe that *G. lamblia*'s ancestors once possessed mitochondria that evolved to become mitosomes. *T. vaginalis*, which causes the sexually transmitted infection vaginitis, is another protozoan parasite that lacks conventional mitochondria. Instead, it possesses hydrogenosomes, mitochondrial-related, double-membrane-bound organelles that produce molecular

hydrogen used in cellular metabolism. Scientists believe that hydrogenosomes, like mitosomes, also evolved from mitochondria.[\[footnote\]](#)

N. Yarlett, J.H.P. Hackstein. "Hydrogenosomes: One Organelle, Multiple Origins." *BioScience* 55 no. 8 (2005):657–658.

Plasma Membrane

The plasma membrane of eukaryotic cells is similar in structure to the prokaryotic plasma membrane in that it is composed mainly of phospholipids forming a bilayer with embedded peripheral and integral proteins ([\[link\]](#)). These membrane components move within the plane of the membrane according to the fluid mosaic model. However, unlike the prokaryotic membrane, eukaryotic membranes contain sterols, including cholesterol, that alter membrane fluidity. Additionally, many eukaryotic cells contain some specialized lipids, including sphingolipids, which are thought to play a role in maintaining membrane stability as well as being involved in signal transduction pathways and cell-to-cell communication.

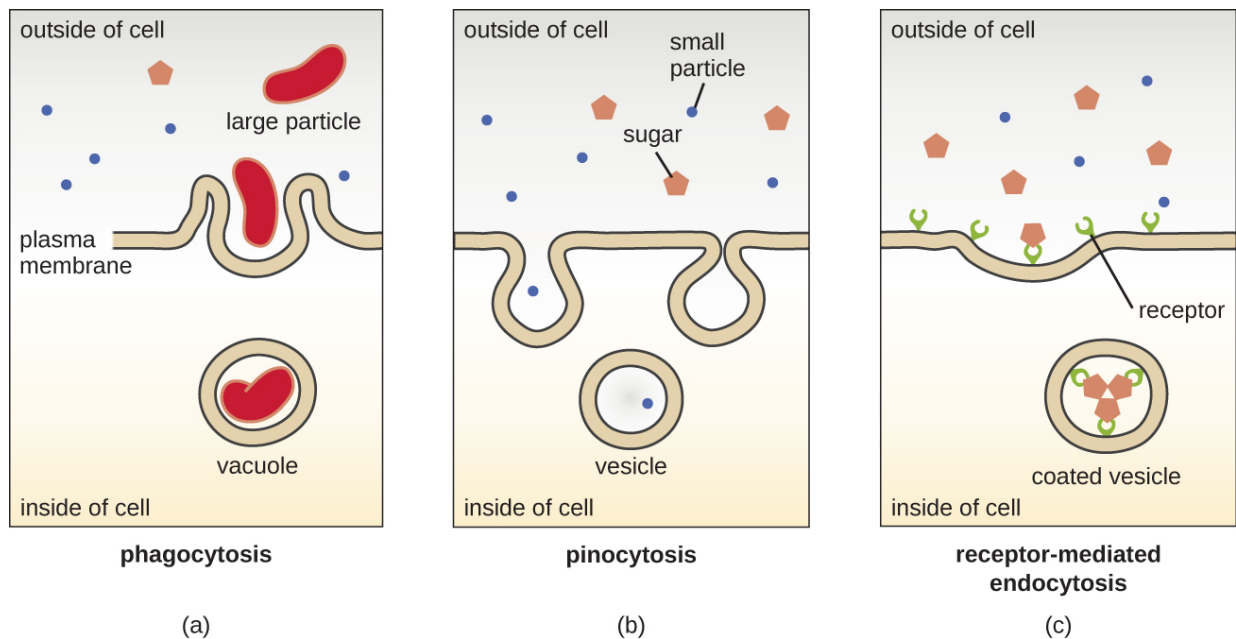


The eukaryotic plasma membrane is composed of a lipid bilayer with many embedded or associated proteins. It contains cholesterol for the maintenance of membrane, as well as glycoproteins and glycolipids that are important in the recognition other cells or pathogens.

Membrane Transport Mechanisms

The processes of simple diffusion, facilitated diffusion, and active transport are used in both eukaryotic and prokaryotic cells. However, eukaryotic cells also have the unique ability to perform various types of **endocytosis**, the uptake of matter through plasma membrane invagination and vacuole/vesicle formation ([\[link\]](#)). A type of endocytosis involving the engulfment of large particles through membrane invagination is called **phagocytosis**, which means “cell eating.” In phagocytosis, particles (or other cells) are enclosed in a pocket within the membrane, which then pinches off from the membrane to form a vacuole that completely surrounds the particle. Another type of endocytosis is called **pinocytosis**, which means “cell drinking.” In pinocytosis, small, dissolved materials and liquids are taken into the cell through small vesicles. Saprophytic fungi, for example, obtain their nutrients from dead and decaying matter largely through pinocytosis.

Receptor-mediated endocytosis is a type of endocytosis that is initiated by specific molecules called ligands when they bind to cell surface receptors on the membrane. Receptor-mediated endocytosis is the mechanism that peptide and amine-derived hormones use to enter cells and is also used by various viruses and bacteria for entry into host cells.



Three variations of endocytosis are shown. (a) In phagocytosis, the cell membrane surrounds the particle and pinches off to form an intracellular vacuole. (b) In pinocytosis, the cell membrane surrounds a small volume of fluid and pinches off, forming a vesicle. (c) In receptor-mediated endocytosis, the uptake of substances is targeted to a specific substance (a ligand) that binds at the receptor on the external cell membrane. (credit: modification of work by Mariana Ruiz Villarreal)

The process by which secretory vesicles release their contents to the cell’s exterior is called **exocytosis**. Vesicles move toward the plasma membrane and then meld with the membrane, ejecting their contents out of the cell. Exocytosis is used by cells to remove waste products and may also be used to release chemical signals that can be taken up by other cells.

Cell Wall

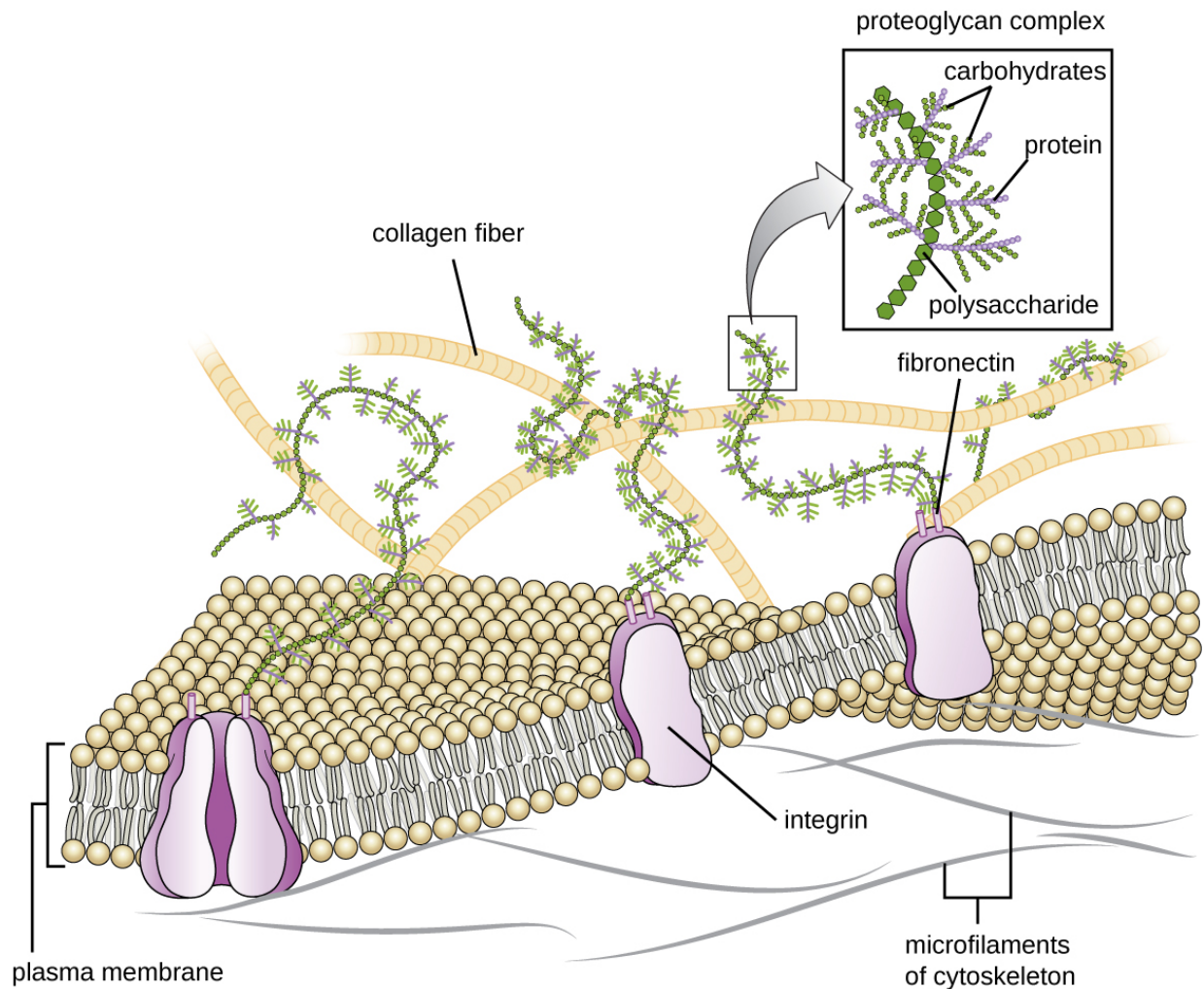
In addition to a plasma membrane, some eukaryotic cells have a cell wall. Cells of fungi, algae, plants, and even some protists have cell walls. Depending upon the type of eukaryotic cell, cell walls can be made of a wide range of materials, including cellulose (fungi and plants); biogenic silica, calcium carbonate, agar, and carrageenan (protists and algae); or chitin (fungi). In general, all cell walls provide structural stability for the cell and protection from environmental stresses such as desiccation, changes in osmotic pressure, and traumatic injury.[\[footnote\]](#)

M. Dudzick. "Protists." OpenStax CNX. November 27, 2013. <http://cnx.org/contents/f7048bb6-e462-459b-805c-ef291cf7049c@1>

Extracellular Matrix

Cells of animals and some protozoans do not have cell walls to help maintain shape and provide structural stability. Instead, these types of eukaryotic cells produce an **extracellular matrix** for this purpose. They secrete a sticky mass of carbohydrates and proteins into the spaces between adjacent cells ([\[link\]](#)). Some protein components assemble into a basement membrane to which the remaining extracellular matrix components adhere. Proteoglycans typically form the bulky mass of the extracellular matrix while fibrous proteins, like collagen, provide strength. Both proteoglycans and collagen are attached to fibronectin proteins, which, in turn, are attached to integrin proteins. These integrin proteins interact with transmembrane proteins in the plasma membranes of eukaryotic cells that lack cell walls.

In animal cells, the extracellular matrix allows cells within tissues to withstand external stresses and transmits signals from the outside of the cell to the inside. The amount of extracellular matrix is quite extensive in various types of connective tissues, and variations in the extracellular matrix can give different types of tissues their distinct properties. In addition, a host cell's extracellular matrix is often the site where microbial pathogens attach themselves to establish infection. For example, *Streptococcus pyogenes*, the bacterium that causes strep throat and various other infections, binds to fibronectin in the extracellular matrix of the cells lining the oropharynx (upper region of the throat).



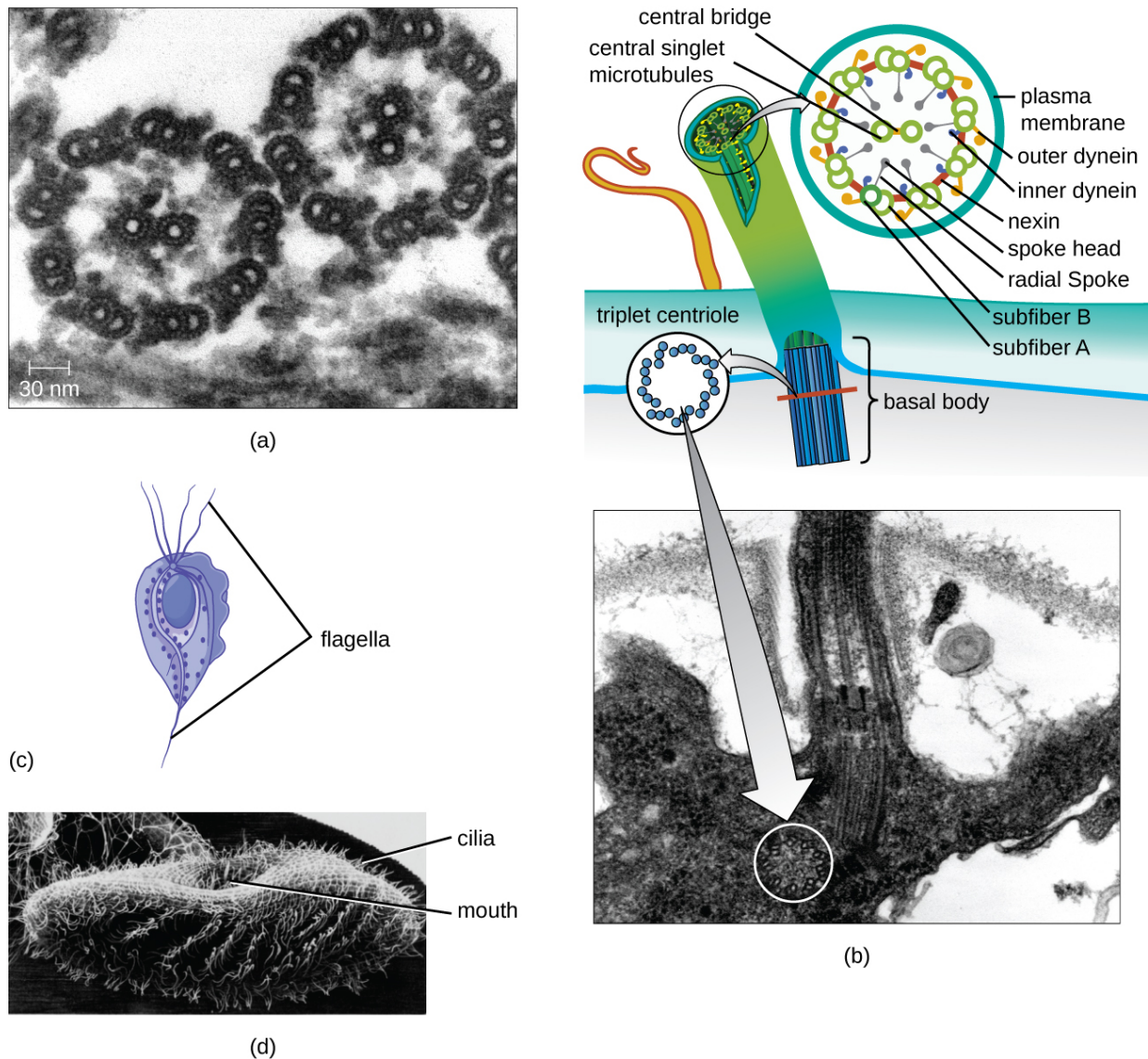
The extracellular matrix is composed of protein and carbohydrate components. It protects cells from physical stresses and transmits signals arriving at the outside edges of the tissue to cells deeper within the tissue.

Flagella and Cilia

Some eukaryotic cells use **flagella** for locomotion; however, eukaryotic flagella are structurally distinct from those found in prokaryotic cells. Whereas the prokaryotic flagellum is a stiff, rotating structure, a eukaryotic flagellum is more like a flexible whip composed of nine parallel pairs of microtubules surrounding a central pair of microtubules. This arrangement is referred to as a 9+2 array ([\[link\]](#)). The parallel microtubules use **dynein** motor proteins to move relative to each other, causing the flagellum to bend.

Cilia (singular: **cilium**) are a similar external structure found in some eukaryotic cells. Unique to eukaryotes, cilia are shorter than flagella and often cover the entire surface of a cell; however, they are structurally similar to flagella (a 9+2 array of microtubules) and use the same mechanism for movement. A structure called a **basal body** is found at the base of each cilium and flagellum. The

basal body, which attaches the cilium or flagellum to the cell, is composed of an array of triplet microtubules similar to that of a centriole but embedded in the plasma membrane. Because of their shorter length, cilia use a rapid, flexible, waving motion. In addition to motility, cilia may have other functions such as sweeping particles past or into cells. For example, ciliated protozoans use the sweeping of cilia to move food particles into their mouthparts, and ciliated cells in the mammalian respiratory tract beat in synchrony to sweep mucus and debris up and out of the lungs ([link](#)).



(a) Eukaryotic flagella and cilia are composed of a 9+2 array of microtubules, as seen in this transmission electron micrograph cross-section. (b) The sliding of these microtubules relative to each other causes a flagellum to bend. (c) An illustration of *Trichomonas vaginalis*, a flagellated protozoan parasite that causes vaginitis. (d) Many protozoans, like this *Paramecium*, have numerous cilia that aid in locomotion as well as in feeding. Note the mouth opening shown here. (credit d: modification of work by University of Vermont/National Institutes of Health)

Note:

- Explain how the cellular envelope of eukaryotic cells compares to that of prokaryotic cells.
- Explain the difference between eukaryotic and prokaryotic flagella.

Note:**Resolution**

Since amoxicillin has not resolved Barbara's case of pneumonia, the PA prescribes another antibiotic, azithromycin, which targets bacterial ribosomes rather than peptidoglycan. After taking the azithromycin as directed, Barbara's symptoms resolve and she finally begins to feel like herself again. Presuming no drug resistance to amoxicillin was involved, and given the effectiveness of azithromycin, the causative agent of Barbara's pneumonia is most likely *Mycoplasma pneumoniae*. Even though this bacterium is a prokaryotic cell, it is not inhibited by amoxicillin because it does not have a cell wall and, therefore, does not make peptidoglycan.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Eukaryotic cells are defined by the presence of a **nucleus** containing the DNA genome and bound by a **nuclear membrane** (or **nuclear envelope**) composed of two lipid bilayers that regulate transport of materials into and out of the nucleus through nuclear pores.
- Eukaryotic cell morphologies vary greatly and may be maintained by various structures, including the cytoskeleton, the cell membrane, and/or the cell wall
- The **nucleolus**, located in the nucleus of eukaryotic cells, is the site of ribosomal synthesis and the first stages of ribosome assembly.
- Eukaryotic cells contain **80S ribosomes** in the rough endoplasmic reticulum (**membrane bound-ribosomes**) and cytoplasm (**free ribosomes**). They contain 70s ribosomes in mitochondria and chloroplasts.
- Eukaryotic cells have evolved an **endomembrane** system, containing membrane-bound organelles involved in transport. These include vesicles, the endoplasmic reticulum, and the Golgi apparatus.
- The **smooth endoplasmic reticulum** plays a role in lipid biosynthesis, carbohydrate metabolism, and detoxification of toxic compounds. The **rough endoplasmic reticulum** contains membrane-bound 80S ribosomes that synthesize proteins destined for the cell membrane
- The **Golgi apparatus** processes proteins and lipids, typically through the addition of sugar molecules, producing glycoproteins or glycolipids, components of the plasma membrane that are used in cell-to-cell communication.
- **Lysosomes** contain digestive enzymes that break down small particles ingested by **endocytosis**, large particles or cells ingested by **phagocytosis**, and damaged intracellular components.
- The **cytoskeleton**, composed of **microfilaments**, **intermediate filaments**, and **microtubules**, provides structural support in eukaryotic cells and serves as a network for transport of intracellular materials.

- **Centrosomes** are microtubule-organizing centers important in the formation of the mitotic spindle in mitosis.
- **Mitochondria** are the site of cellular respiration. They have two membranes: an outer membrane and an inner membrane with cristae. The mitochondrial matrix, within the inner membrane, contains the mitochondrial DNA, 70S ribosomes, and metabolic enzymes.
- The plasma membrane of eukaryotic cells is structurally similar to that found in prokaryotic cells, and membrane components move according to the fluid mosaic model. However, eukaryotic membranes contain sterols, which alter membrane fluidity, as well as glycoproteins and glycolipids, which help the cell recognize other cells and infectious particles.
- In addition to active transport and passive transport, eukaryotic cell membranes can take material into the cell via **endocytosis**, or expel matter from the cell via **exocytosis**.
- Cells of fungi, algae, plants, and some protists have a **cell wall**, whereas cells of animals and some protozoans have a sticky **extracellular matrix** that provides structural support and mediates cellular signaling.
- Eukaryotic flagella are structurally distinct from prokaryotic flagella but serve a similar purpose (locomotion). **Cilia** are structurally similar to eukaryotic flagella, but shorter; they may be used for locomotion, feeding, or movement of extracellular particles.

Multiple Choice

Exercise:

Problem: Which of the following organelles is not part of the endomembrane system?

- A. endoplasmic reticulum
- B. Golgi apparatus
- C. lysosome
- D. peroxisome

Solution:

D

Exercise:

Problem: Which type of cytoskeletal fiber is important in the formation of the nuclear lamina?

- A. microfilaments
- B. intermediate filaments
- C. microtubules
- D. fibronectin

Solution:

B

Exercise:

Problem: Sugar groups may be added to proteins in which of the following?

- A. smooth endoplasmic reticulum
- B. rough endoplasmic reticulum
- C. Golgi apparatus
- D. lysosome

Solution:

C

Exercise:

Problem:

Which of the following structures of a eukaryotic cell is not likely derived from endosymbiotic bacterium?

- A. mitochondrial DNA
- B. mitochondrial ribosomes
- C. inner membrane
- D. outer membrane

Solution:

D

Exercise:

Problem:

Which type of nutrient uptake involves the engulfment of small dissolved molecules into vesicles?

- A. active transport
- B. pinocytosis
- C. receptor-mediated endocytosis
- D. facilitated diffusion

Solution:

B

Exercise:

Problem: Which of the following is not composed of microtubules?

- A. desmosomes
- B. centrioles
- C. eukaryotic flagella
- D. eukaryotic cilia

Solution:

A

True/False

Exercise:

Problem:

Mitochondria in eukaryotic cells contain ribosomes that are structurally similar to those found in prokaryotic cells.

Solution:

True

Fill in the Blank

Exercise:

Problem:

Peroxisomes typically produce _____, a harsh chemical that helps break down molecules.

Solution:

hydrogen peroxide

Exercise:

Problem: Microfilaments are composed of _____ monomers.

Solution:

actin

Short Answer

Exercise:

Problem:

What existing evidence supports the theory that mitochondria are of prokaryotic origin?

Exercise:

Problem: Why do eukaryotic cells require an endomembrane system?

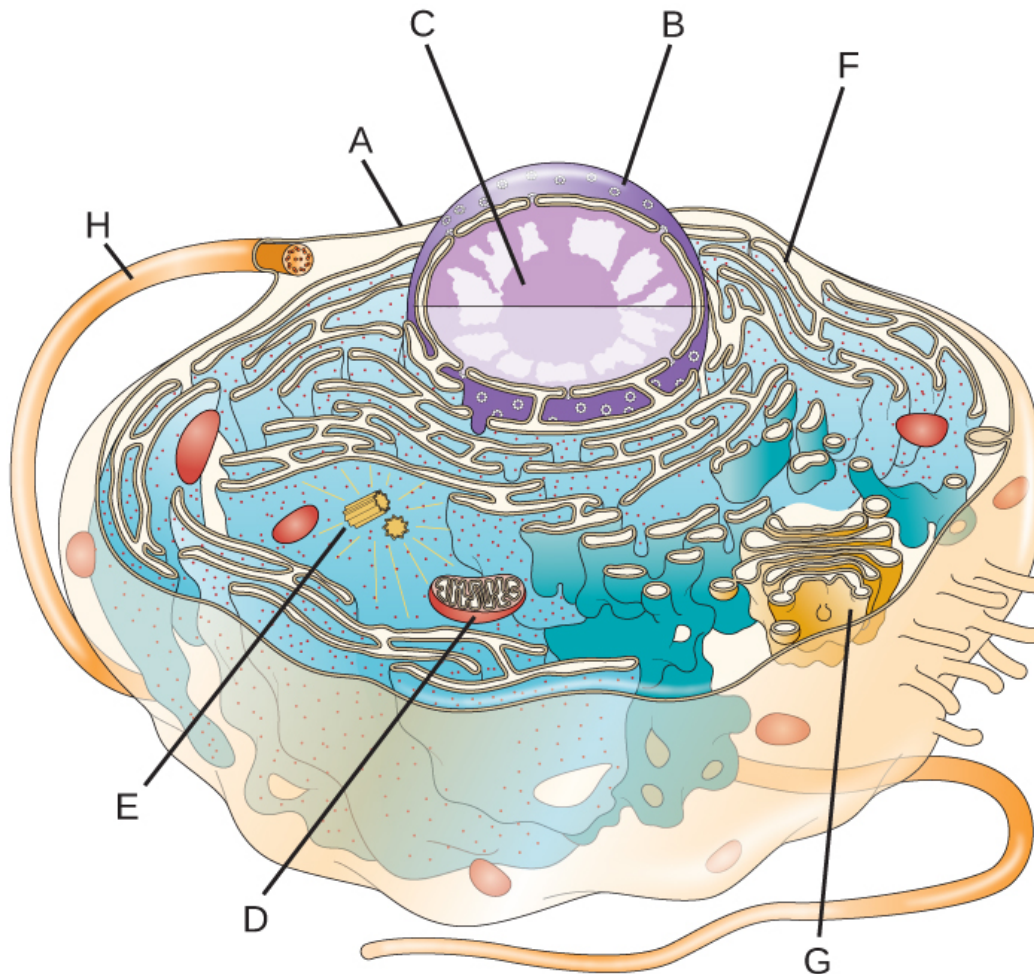
Exercise:

Problem: Name at least two ways that prokaryotic flagella are different from eukaryotic flagella.

Critical Thinking

Exercise:

Problem: Label the lettered parts of this eukaryotic cell.



Exercise:

Problem:

How are peroxisomes more like mitochondria than like the membrane-bound organelles of the endomembrane system? How do they differ from mitochondria?

Exercise:

Problem: Why must the functions of both lysosomes and peroxisomes be compartmentalized?

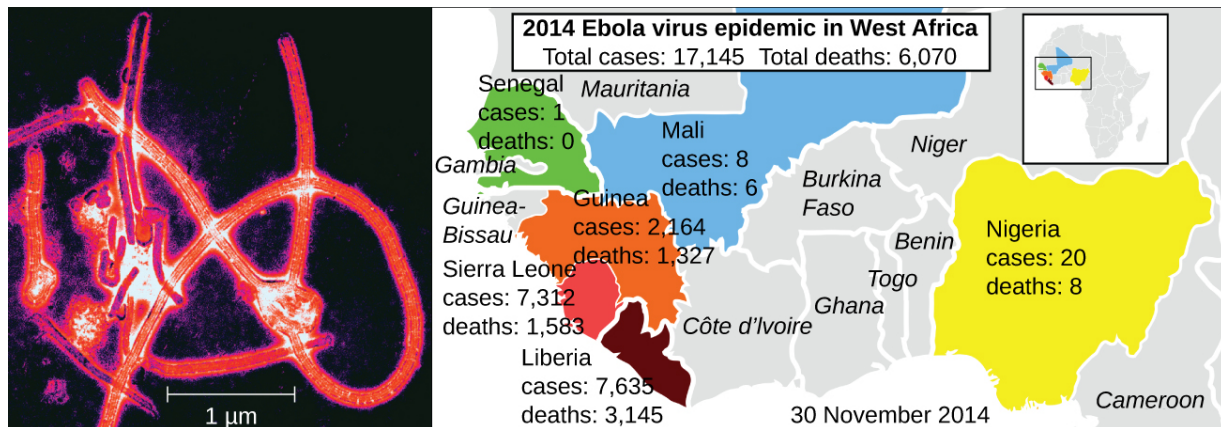
Introduction

class="introduction"

The year
2014 saw
the first
large-scale
outbreak of
Ebola virus
(electron
micrograph,
left) in
human
populations
in West
Africa

(right). Such
epidemics
are now
widely
reported and
documented
, but viral
epidemics
are sure to
have
plagued
human
populations
since the
origin of our
species.

(credit left:
modificatio
n of work
by Thomas
W. Geisbert)



Public health measures in the developed world have dramatically reduced mortality from viral epidemics. But when epidemics do occur, they can spread quickly with global air travel. In 2009, an outbreak of H1N1 influenza spread across various continents. In early 2014, cases of Ebola in Guinea led to a massive epidemic in western Africa. This included the case of an infected man who traveled to the United States, sparking fears the epidemic might spread beyond Africa.

Until the late 1930s and the advent of the electron microscope, no one had seen a virus. Yet treatments for preventing or curing viral infections were used and developed long before that. Historical records suggest that by the 17th century, and perhaps earlier, inoculation (also known as variolation) was being used to prevent the viral disease smallpox in various parts of the world. By the late 18th century, Englishman Edward Jenner was inoculating patients with cowpox to prevent smallpox, a technique he coined *vaccination*.[\[footnote\]](#)

S. Riedel “Edward Jenner and the History of Smallpox and Vaccination.” *Baylor University Medical Center Proceedings* 18, no. 1 (January 2005): 21–25.

Today, the structure and genetics of viruses are well defined, yet new discoveries continue to reveal their complexities. In this chapter, we will learn about the structure, classification, and cultivation of viruses, and how they impact their hosts. In addition, we will learn about other infective particles such as viroids and prions.

Viruses

LEARNING OBJECTIVES

- Describe the general characteristics of viruses as pathogens
- Describe viral genomes
- Describe the general characteristics of viral life cycles
- Differentiate among bacteriophages, plant viruses, and animal viruses
- Describe the characteristics used to identify viruses as obligate intracellular parasites

Note:

Part 1

David, a 45-year-old journalist, has just returned to the U.S. from travels in Russia, China, and Africa. He is not feeling well, so he goes to his general practitioner complaining of weakness in his arms and legs, fever, headache, noticeable agitation, and minor discomfort. He thinks it may be related to a dog bite he suffered while interviewing a Chinese farmer. He is experiencing some prickling and itching sensations at the site of the bite wound, but he tells the doctor that the dog seemed healthy and that he had not been concerned until now. The doctor ordered a culture and sensitivity test to rule out bacterial infection of the wound, and the results came back negative for any possible pathogenic bacteria.

- Based on this information, what additional tests should be performed on the patient?
- What type of treatment should the doctor recommend?

Jump to the [next](#) Clinical Focus box.

Despite their small size, which prevented them from being seen with light microscopes, the discovery of a filterable component smaller than a bacterium that

causes tobacco mosaic disease (TMD) dates back to 1892.[\[footnote\]](#) At that time, Dmitri Ivanovski, a Russian botanist, discovered the source of TMD by using a porcelain filtering device first invented by Charles Chamberland and Louis Pasteur in Paris in 1884. Porcelain Chamberland filters have a pore size of 0.1 μm , which is small enough to remove all bacteria $\geq 0.2 \mu\text{m}$ from any liquids passed through the device. An extract obtained from TMD-infected tobacco plants was made to determine the cause of the disease. Initially, the source of the disease was thought to be bacterial. It was surprising to everyone when Ivanovski, using a Chamberland filter, found that the cause of TMD was not removed after passing the extract through the porcelain filter. So if a bacterium was not the cause of TMD, what could be causing the disease? Ivanovski concluded the cause of TMD must be an extremely small bacterium or bacterial spore. Other scientists, including Martinus Beijerinck, continued investigating the cause of TMD. It was Beijerinck, in 1899, who eventually concluded the causative agent was not a bacterium but, instead, possibly a chemical, like a biological poison we would describe today as a toxin. As a result, the word *virus*, Latin for poison, was used to describe the cause of TMD a few years after Ivanovski's initial discovery. Even though he was not able to see the virus that caused TMD, and did not realize the cause was not a bacterium, Ivanovski is credited as the original discoverer of viruses and a founder of the field of virology.

H. Lecoq. “[Discovery of the First Virus, the Tobacco Mosaic Virus: 1892 or 1898?].” *Comptes Rendus de l’Academie des Sciences – Serie III – Sciences de la Vie* 324, no. 10 (2001): 929–933.

Today, we can see viruses using electron microscopes ([\[link\]](#)) and we know much more about them. Viruses are distinct biological entities; however, their evolutionary origin is still a matter of speculation. In terms of taxonomy, they are not included in the tree of life because they are **acellular** (not consisting of cells). In order to survive and reproduce, viruses must infect a cellular host, making them obligate intracellular parasites. The genome of a virus enters a host cell and directs the production of the viral components, proteins and nucleic acids, needed to form new virus particles called **virions**. New virions are made in the host cell by assembly of viral components. The new virions transport the viral genome to another host cell to carry out another round of infection. [\[link\]](#) summarizes the properties of viruses.



Characteristics of Viruses

Characteristics of Viruses

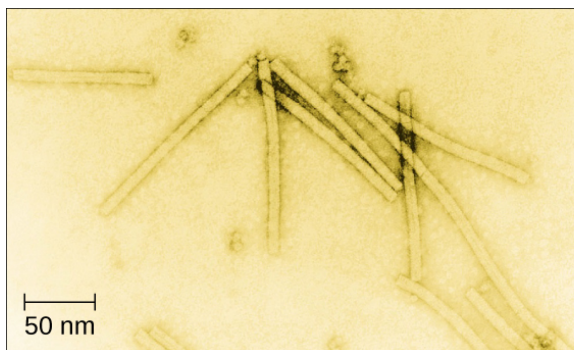
Infectious, acellular pathogens

Obligate intracellular parasites with host and cell-type specificity

DNA or RNA genome (never both)

Genome is surrounded by a protein capsid and, in some cases, a phospholipid membrane studded with viral glycoproteins

Lack genes for many products needed for successful reproduction, requiring exploitation of host-cell genomes to reproduce



(a)



(b)

(a) Tobacco mosaic virus (TMV) viewed with transmission electron microscope. (b) Plants infected with tobacco mosaic disease (TMD), caused by TMV. (credit a: modification of work by USDA Agricultural Research Service—scale-bar data from Matt Russell; credit b: modification of work by USDA Forest Service, Department of Plant Pathology Archive North Carolina State University)

Note:

- Why was the first virus investigated mistaken for a toxin?

Hosts and Viral Transmission

Viruses can infect every type of host cell, including those of plants, animals, fungi, protists, bacteria, and archaea. Most viruses will only be able to infect the cells of one or a few species of organism. This is called the **host range**. However, having a wide host range is not common and viruses will typically only infect specific hosts and only specific cell types within those hosts. The viruses that infect bacteria are called **bacteriophages**, or simply phages. The word *phage* comes from the Greek word for devour. Other viruses are just identified by their host group, such as animal or plant viruses. Once a cell is infected, the effects of the virus can vary depending on the type of virus. Viruses may cause abnormal growth of the cell or cell death, alter the cell's genome, or cause little noticeable effect in the cell.

Viruses can be transmitted through direct contact, indirect contact with fomites, or through a **vector**: an animal that transmits a pathogen from one host to another.

Arthropods such as mosquitoes, ticks, and flies, are typical vectors for viral diseases, and they may act as **mechanical vectors** or **biological vectors**.

Mechanical transmission occurs when the arthropod carries a viral pathogen on the outside of its body and transmits it to a new host by physical contact. Biological transmission occurs when the arthropod carries the viral pathogen inside its body and transmits it to the new host through biting.

In humans, a wide variety of viruses are capable of causing various infections and diseases. Some of the deadliest emerging pathogens in humans are viruses, yet we have few treatments or drugs to deal with viral infections, making them difficult to eradicate.

Viruses that can be transmitted from an animal host to a human host can cause zoonoses. For example, the avian influenza virus originates in birds, but can cause disease in humans. Reverse zoonoses are caused by infection of an animal by a virus that originated in a human.

Note:

Fighting Bacteria with Viruses

The emergence of superbugs, or multidrug resistant bacteria, has become a major challenge for pharmaceutical companies and a serious health-care problem.

According to a 2013 report by the US Centers for Disease Control and Prevention

(CDC), more than 2 million people are infected with drug-resistant bacteria in the US annually, resulting in at least 23,000 deaths. [\[footnote\]](#) The continued use and overuse of antibiotics will likely lead to the evolution of even more drug-resistant strains.

US Department of Health and Human Services, Centers for Disease Control and Prevention. “Antibiotic Resistance Threats in the United States, 2013.”

<http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf> (accessed September 22, 2015).

One potential solution is the use of phage therapy, a procedure that uses bacteria-killing viruses (bacteriophages) to treat bacterial infections. Phage therapy is not a new idea. The discovery of bacteriophages dates back to the early 20th century, and phage therapy was first used in Europe in 1915 by the English bacteriologist Frederick Twort. [\[footnote\]](#) However, the subsequent discovery of penicillin and other antibiotics led to the near abandonment of this form of therapy, except in the former Soviet Union and a few countries in Eastern Europe. Interest in phage therapy outside of the countries of the former Soviet Union is only recently re-emerging because of the rise in antibiotic-resistant bacteria. [\[footnote\]](#)

M. Clokie et al. “Phages in Nature.” *Bacteriophage* 1, no. 1 (2011): 31–45.

A. Sulakvelidze et al. “Bacteriophage Therapy.” *Antimicrobial Agents and Chemotherapy* 45, no. 3 (2001): 649–659.

Phage therapy has some advantages over antibiotics in that phages kill only one specific bacterium, whereas antibiotics kill not only the pathogen but also beneficial bacteria of the normal microbiota. Development of new antibiotics is also expensive for drug companies and for patients, especially for those who live in countries with high poverty rates.

Phages have also been used to prevent food spoilage. In 2006, the US Food and Drug Administration approved the use of a solution containing six bacteriophages that can be sprayed on lunch meats such as bologna, ham, and turkey to kill *Listeria monocytogenes*, a bacterium responsible for listeriosis, a form of food poisoning. Some consumers have concerns about the use of phages on foods, however, especially given the rising popularity of organic products. Foods that have been treated with phages must declare “bacteriophage preparation” in the list of ingredients or include a label declaring that the meat has been “treated with antimicrobial solution to reduce microorganisms.” [\[footnote\]](#)

US Food and Drug Administration. “FDA Approval of *Listeria*-specific Bacteriophage Preparation on Ready-to-Eat (RTE) Meat and Poultry Products.”

<http://www.fda.gov/food/ingredientspackaginglabeling/ucm083572.htm> (accessed September 22, 2015).

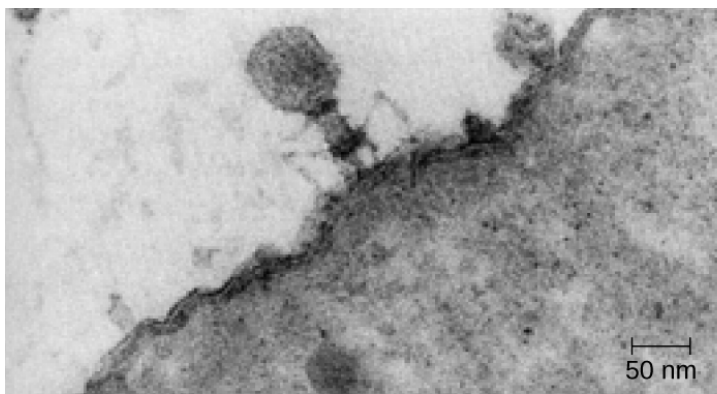
Note:

- Why do humans not have to be concerned about the presence of bacteriophages in their food?
- What are three ways that viruses can be transmitted between hosts?

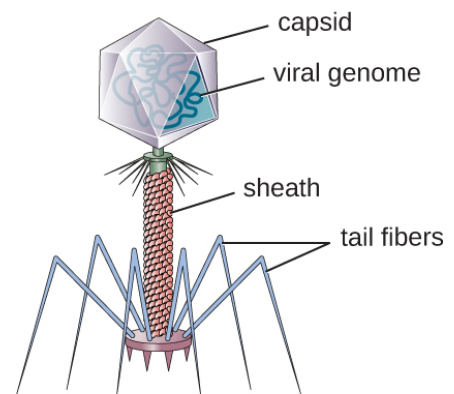
Viral Structures

In general, virions (viral particles) are small and cannot be observed using a regular light microscope. They are much smaller than prokaryotic and eukaryotic cells; this is an adaptation allowing viruses to infect these larger cells (see [\[link\]](#)). The size of a virion can range from 20 nm for small viruses up to 900 nm for typical, large viruses (see [\[link\]](#)). Recent discoveries, however, have identified new giant viral species, such as *Pandoravirus salinus* and *Pithovirus sibericum*, with sizes approaching that of a bacterial cell.[\[footnote\]](#)

N. Philippe et al. “Pandoraviruses: Amoeba Viruses with Genomes up to 2.5 Mb Reaching that of Parasitic Eukaryotes.” *Science* 341, no. 6143 (2013): 281–286.

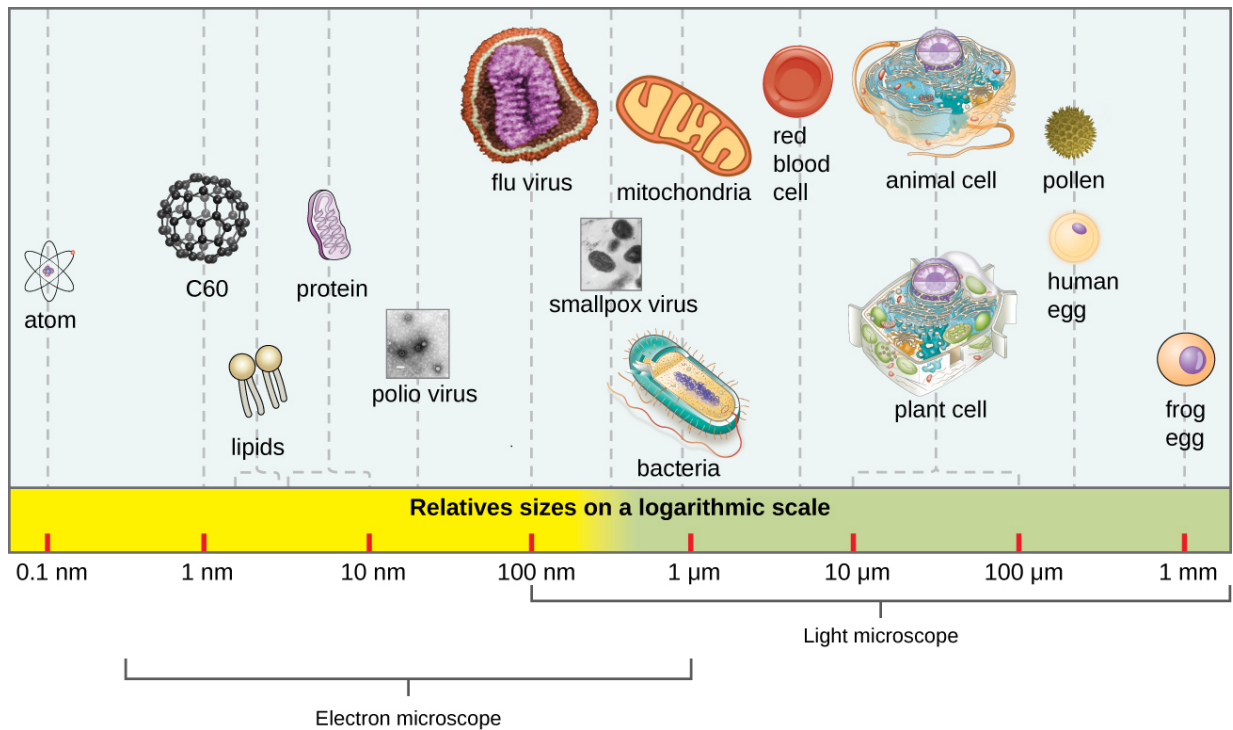


(a)



(b)

(a) In this transmission electron micrograph, a bacteriophage (a virus that infects bacteria) is dwarfed by the bacterial cell it infects. (b) An illustration of the bacteriophage in the micrograph. (credit a: modification of work by U.S. Department of Energy, Office of Science, LBL, PBD)



The size of a virus is small relative to the size of most bacterial and eukaryotic cells and their organelles.

In 1935, after the development of the electron microscope, Wendell Stanley was the first scientist to crystallize the structure of the tobacco mosaic virus and discovered that it is composed of RNA and protein. In 1943, he isolated *Influenza B virus*, which contributed to the development of an influenza (flu) vaccine. Stanley's discoveries unlocked the mystery of the nature of viruses that had been puzzling scientists for over 40 years and his contributions to the field of virology led to him being awarded the Nobel Prize in 1946.

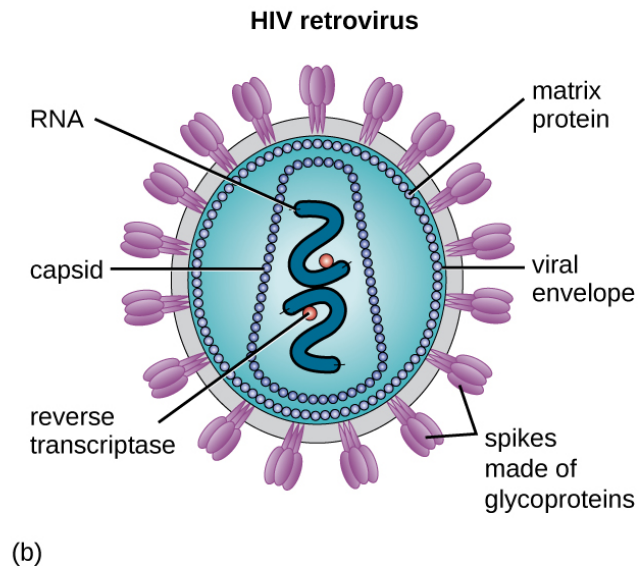
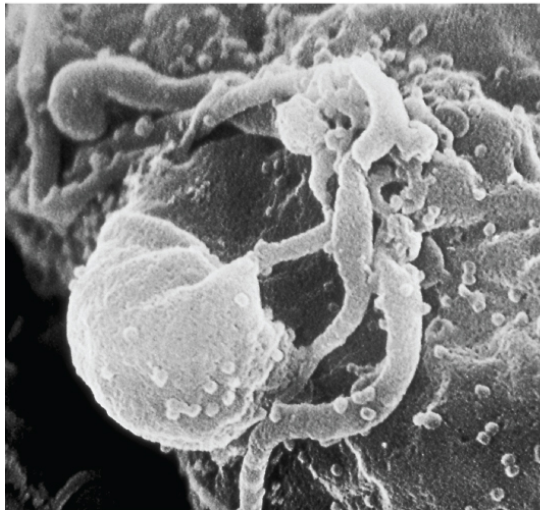
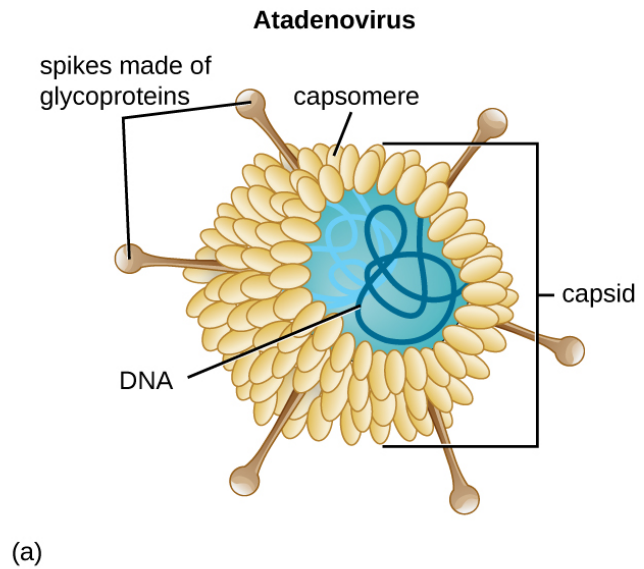
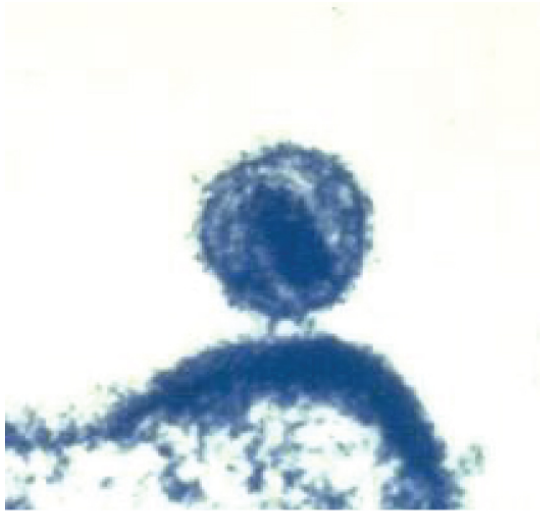
As a result of continuing research into the nature of viruses, we now know they consist of a nucleic acid (either RNA or DNA, but never both) surrounded by a protein coat called a **capsid** (see [\[link\]](#)). The interior of the capsid is not filled with cytosol, as in a cell, but instead it contains the bare necessities in terms of genome and enzymes needed to direct the synthesis of new virions. Each capsid is composed of protein subunits called **capsomeres** made of one or more different types of capsomere proteins that interlock to form the closely packed capsid.

There are two categories of viruses based on general composition. Viruses formed from only a nucleic acid and capsid are called **naked viruses** or **nonenveloped**

viruses. Viruses formed with a nucleic-acid packed capsid surrounded by a lipid layer are called **enveloped viruses** (see [\[link\]](#)). The **viral envelope** is a small portion of phospholipid membrane obtained as the virion buds from a host cell. The viral envelope may either be intracellular or cytoplasmic in origin.

Extending outward and away from the capsid on some naked viruses and enveloped viruses are protein structures called **spikes**. At the tips of these spikes are structures that allow the virus to attach and enter a cell, like the influenza virus hemagglutinin spikes (H) or enzymes like the neuraminidase (N) influenza virus spikes that allow the virus to detach from the cell surface during release of new virions. Influenza viruses are often identified by their H and N spikes. For example, H1N1 influenza viruses were responsible for the pandemics in 1918 and 2009,[\[footnote\]](#) H2N2 for the pandemic in 1957, and H3N2 for the pandemic in 1968.

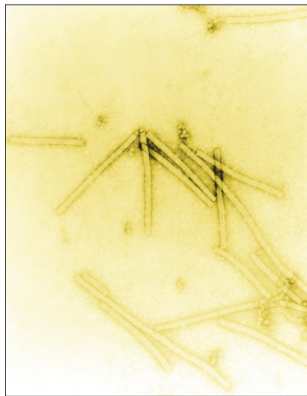
J. Cohen. “What’s Old Is New: 1918 Virus Matches 2009 H1N1 Strain. *Science* 327, no. 5973 (2010): 1563–1564.



(a) The naked adenovirus uses spikes made of glycoproteins from its capsid to bind to host cells. (b) The enveloped human immunodeficiency virus uses spikes made of glycoproteins embedded in its envelope to bind to host cells
 (credit a “micrograph”: modification of work by NIAID; credit b
 “micrograph”: modification of work by Centers for Disease Control and Prevention)

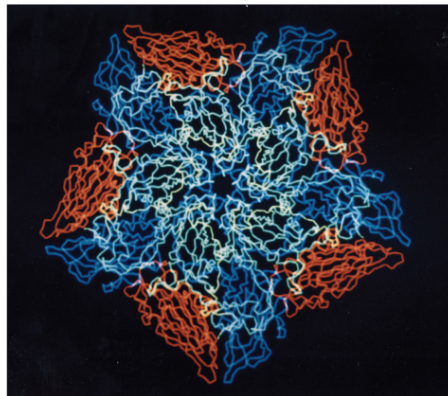
Viruses vary in the shape of their capsids, which can be either **helical**, **polyhedral**, or **complex**. A helical capsid forms the shape of tobacco mosaic virus (TMV), a

naked helical virus, and Ebola virus, an enveloped helical virus. The capsid is cylindrical or rod shaped, with the genome fitting just inside the length of the capsid. Polyhedral capsids form the shapes of poliovirus and rhinovirus, and consist of a nucleic acid surrounded by a polyhedral (many-sided) capsid in the form of an icosahedron. An **icosahedral** capsid is a three-dimensional, 20-sided structure with 12 vertices. These capsids somewhat resemble a soccer ball. Both helical and polyhedral viruses can have envelopes. Viral shapes seen in certain types of bacteriophages, such as T4 phage, and poxviruses, like vaccinia virus, may have features of both polyhedral and helical viruses so they are described as a complex viral shape (see [\[link\]](#)). In the bacteriophage complex form, the genome is located within the polyhedral head and the **sheath** connects the head to the **tail fibers** and **tail pins** that help the virus attach to receptors on the host cell's surface. Poxviruses that have complex shapes are often brick shaped, with intricate surface characteristics not seen in the other categories of capsid.



Tobacco mosaic virus

Helical



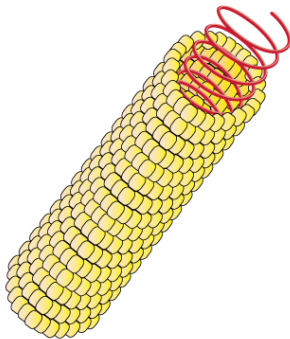
Human rhinovirus HRV14

Icosahedral

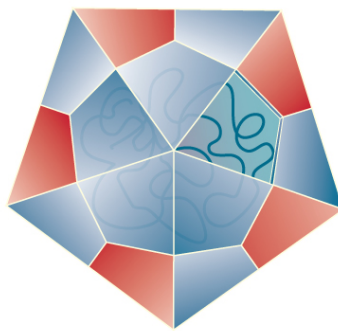


Variola virus

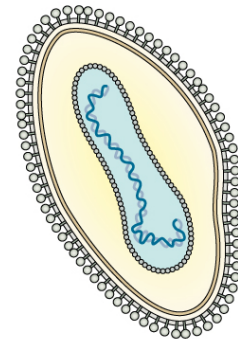
Complex



(a)



(b)



(c)

Viral capsids can be (a) helical, (b) polyhedral, or (c) have a complex shape.

(credit a “micrograph”: modification of work by USDA ARS; credit b “micrograph”: modification of work by U.S. Department of Energy)

Note:

- Which types of viruses have spikes?

Classification and Taxonomy of Viruses

Although viruses are not classified in the three domains of life, their numbers are great enough to require classification. Since 1971, the International Union of Microbiological Societies Virology Division has given the task of developing, refining, and maintaining a universal virus taxonomy to the International Committee on Taxonomy of Viruses (ICTV). Since viruses can mutate so quickly, it can be difficult to classify them into a genus and a species epithet using the binomial nomenclature system. Thus, the ICTV’s viral nomenclature system classifies viruses into families and genera based on viral genetics, chemistry, morphology, and mechanism of multiplication. To date, the ICTV has classified known viruses in seven orders, 96 families, and 350 genera. Viral family names end in *-viridae* (e.g, *Parvoviridae*) and genus names end in *-virus* (e.g., *Parvovirus*). The names of viral orders, families, and genera are all italicized. When referring to a viral species, we often use a genus and species epithet such as *Pandoravirus dulcis* or *Pandoravirus salinus*.

The Baltimore classification system is an alternative to ICTV nomenclature. The Baltimore system classifies viruses according to their genomes (DNA or RNA, single versus double stranded, and mode of replication). This system thus creates seven groups of viruses that have common genetics and biology.

Note:

Explore the latest virus [taxonomy](#) at the ICTV website.

Aside from formal systems of nomenclature, viruses are often informally grouped into categories based on chemistry, morphology, or other characteristics they share in common. Categories may include naked or enveloped structure, single-stranded (ss) or double-stranded (ds) DNA or ss or ds RNA genomes, segmented or nonsegmented genomes, and positive-strand (+) or negative-strand (–) RNA. For example, herpes viruses can be classified as a dsDNA enveloped virus; human immunodeficiency virus (HIV) is a +ssRNA enveloped virus, and tobacco mosaic virus is a +ssRNA virus. Other characteristics such as host specificity, tissue specificity, capsid shape, and special genes or enzymes may also be used to describe groups of similar viruses. [\[link\]](#) lists some of the most common viruses that are human pathogens by genome type.

Common Pathogenic Viruses			
Genome	Family	Example Virus	Clinical Features
dsDNA, enveloped	<i>Poxviridae</i>	<i>Orthopoxvirus</i>	Skin papules, pustules, lesions
	<i>Poxviridae</i>	<i>Parapoxvirus</i>	Skin lesions
	<i>Herpesviridae</i>	<i>Simplexvirus</i>	Cold sores, genital herpes, sexually transmitted disease
dsDNA, naked	<i>Adenoviridae</i>	<i>Atadenovirus</i>	Respiratory infection (common cold)

Common Pathogenic Viruses			
Genome	Family	Example Virus	Clinical Features
	<i>Papillomaviridae</i>	<i>Papillomavirus</i>	Genital warts, cervical, vulvar, or vaginal cancer
	<i>Reoviridae</i>	<i>Reovirus</i>	Gastroenteritis severe diarrhea (stomach flu)
ssDNA, naked	<i>Parvoviridae</i>	<i>Adeno-associated dependoparvovirus A</i>	Respiratory tract infection
	<i>Parvoviridae</i>	<i>Adeno-associated dependoparvovirus B</i>	Respiratory tract infection
dsRNA, naked	<i>Reoviridae</i>	<i>Rotavirus</i>	Gastroenteritis
+ssRNA, naked	<i>Picornaviridae</i>	<i>Enterovirus C</i>	Poliomyelitis
	<i>Picornaviridae</i>	<i>Rhinovirus</i>	Upper respiratory tract infection (common cold)
	<i>Picornaviridae</i>	<i>Hepatovirus</i>	Hepatitis
+ssRNA, enveloped	<i>Togaviridae</i>	<i>Alphavirus</i>	Encephalitis, hemorrhagic fever

Common Pathogenic Viruses			
Genome	Family	Example Virus	Clinical Features
	<i>Togaviridae</i>	<i>Rubivirus</i>	Rubella
	<i>Retroviridae</i>	<i>Lentivirus</i>	Acquired immune deficiency syndrome (AIDS)
-ssRNA, enveloped	<i>Filoviridae</i>	<i>Zaire Ebolavirus</i>	Hemorrhagic fever
	<i>Orthomyxoviridae</i>	<i>Influenzavirus A, B, C</i>	Flu
	<i>Rhabdoviridae</i>	<i>Lyssavirus</i>	Rabies

Note:

- What are the types of virus genomes?

Classification of Viral Diseases

While the ICTV has been tasked with the biological classification of viruses, it has also played an important role in the classification of diseases caused by viruses. To facilitate the tracking of virus-related human diseases, the ICTV has created classifications that link to the International Classification of Diseases (ICD), the standard taxonomy of disease that is maintained and updated by the World Health Organization (WHO). The ICD assigns an alphanumeric code of up to six characters to every type of viral infection, as well as all other types of diseases, medical conditions, and causes of death. This ICD code is used in conjunction with two other coding systems (the Current Procedural Terminology, and the Healthcare

Common Procedure Coding System) to categorize patient conditions for treatment and insurance reimbursement.

For example, when a patient seeks treatment for a viral infection, ICD codes are routinely used by clinicians to order laboratory tests and prescribe treatments specific to the virus suspected of causing the illness. This ICD code is then used by medical laboratories to identify tests that must be performed to confirm the diagnosis. The ICD code is used by the health-care management system to verify that all treatments and laboratory work performed are appropriate for the given virus. Medical coders use ICD codes to assign the proper code for procedures performed, and medical billers, in turn, use this information to process claims for reimbursement by insurance companies. Vital-records keepers use ICD codes to record cause of death on death certificates, and epidemiologists used ICD codes to calculate morbidity and mortality statistics.

Note:

- Identify two locations where you would likely find an ICD code.

Note:

Part 2

David's doctor was concerned that his symptoms included prickling and itching at the site of the dog bite; these sensations could be early symptoms of rabies.

Several tests are available to diagnose rabies in live patients, but no single antemortem test is adequate. The doctor decided to take samples of David's blood, saliva, and skin for testing. The skin sample was taken from the nape of the neck (posterior side of the neck near the hairline). It was about 6-mm long and contained at least 10 hair follicles, including the superficial cutaneous nerve. An immunofluorescent staining technique was used on the skin biopsy specimen to detect rabies antibodies in the cutaneous nerves at the base of the hair follicles. A test was also performed on a serum sample from David's blood to determine whether any antibodies for the rabies virus had been produced.

Meanwhile, the saliva sample was used for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, a test that can detect the presence of viral nucleic acid (RNA). The blood tests came back positive for the presence of rabies virus antigen, prompting David's doctor to prescribe prophylactic treatment. David is

given a series of intramuscular injections of human rabies immunoglobulin along with a series of rabies vaccines.

- Why does the immunofluorescent technique look for rabies antibodies rather than the rabies virus itself?
- If David has contracted rabies, what is his prognosis?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Viruses are generally ultramicroscopic, typically from 20 nm to 900 nm in length. Some large viruses have been found.
- **Virions** are acellular and consist of a nucleic acid, DNA or RNA, but not both, surrounded by a protein **capsid**. There may also be a phospholipid membrane surrounding the capsid.
- Viruses are obligate intracellular parasites.
- Viruses are known to infect various types of cells found in plants, animals, fungi, protists, bacteria, and archaea. Viruses typically have limited **host ranges** and infect specific cell types.
- Viruses may have **helical**, **polyhedral**, or **complex** shapes.
- Classification of viruses is based on morphology, type of nucleic acid, host range, cell specificity, and enzymes carried within the virion.
- Like other diseases, viral diseases are classified using ICD codes.

Multiple Choice

Exercise:

Problem:

The component(s) of a virus that is/are extended from the envelope for attachment is/are the:

- A. capsomeres
- B. spikes
- C. nucleic acid
- D. viral whiskers

Solution:

B

Exercise:

Problem: Which of the following does a virus lack? Select all that apply.

- A. ribosomes
- B. metabolic processes
- C. nucleic acid
- D. glycoprotein

Solution:

A and B

Exercise:

Problem: The envelope of a virus is derived from the host's

- A. nucleic acids
- B. membrane structures
- C. cytoplasm
- D. genome

Solution:

B

Exercise:

Problem:

In naming viruses, the family name ends with _____ and genus name ends with _____.

- A. *-virus*; *-viridae*
- B. *-viridae*; *-virus*
- C. *-virion*; *virus*
- D. *-virus*; *virion*

Solution:

B

Exercise:

Problem: What is another name for a nonenveloped virus?

- A. enveloped virus
- B. provirus
- C. naked virus
- D. latent virus

Solution:

C

True/False

Exercise:

Problem:

True or False: Scientists have identified viruses that are able to infect fungal cells.

Solution:

True

Fill in the Blank

Exercise:

Problem:

A virus that infects a bacterium is called a/an _____.

Solution:

bacteriophage

Exercise:

Problem:

A/an _____ virus possesses characteristics of both a polyhedral and helical virus.

Solution:

complex

Exercise:

Problem:

A virus containing only nucleic acid and a capsid is called a/an _____ virus or _____ virus.

Solution:

naked or nonenveloped

Exercise:

Problem:

The _____ on the bacteriophage allow for binding to the bacterial cell.

Solution:

tail fibers

Short Answer

Exercise:

Problem:

Discuss the geometric differences among helical, polyhedral, and complex viruses.

Exercise:

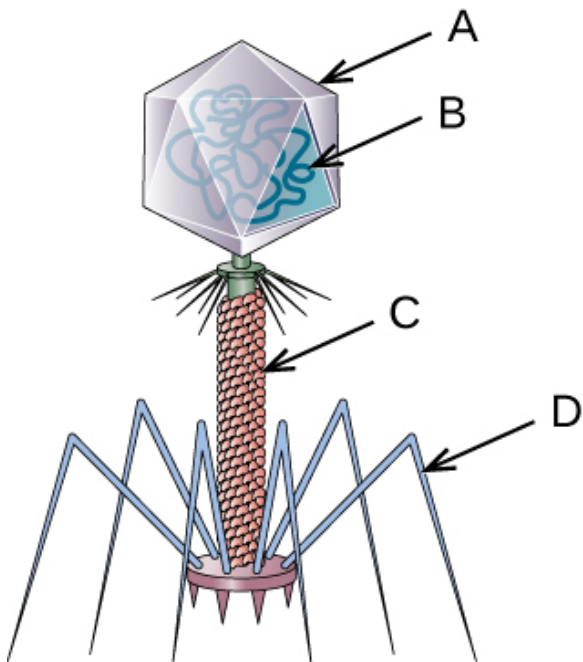
Problem:

What was the meaning of the word “virus” in the 1880s and why was it used to describe the cause of tobacco mosaic disease?

Critical Thinking

Exercise:

Problem: Name each labeled part of the illustrated bacteriophage.



Exercise:

Problem:

In terms of evolution, which do you think arises first? The virus or the host? Explain your answer.

Exercise:

Problem:

Do you think it is possible to create a virus in the lab? Imagine that you are a mad scientist. Describe how you would go about creating a new virus.

The Viral Life Cycle

LEARNING OBJECTIVES

- Describe the lytic and lysogenic life cycles
- Describe the replication process of animal viruses
- Describe unique characteristics of retroviruses and latent viruses
- Discuss human viruses and their virus-host cell interactions
- Explain the process of transduction
- Describe the replication process of plant viruses

All viruses depend on cells for reproduction and metabolic processes. By themselves, viruses do not encode for all of the enzymes necessary for viral replication. But within a host cell, a virus can commandeer cellular machinery to produce more viral particles. Bacteriophages replicate only in the cytoplasm, since prokaryotic cells do not have a nucleus or organelles. In eukaryotic cells, most DNA viruses can replicate inside the nucleus, with an exception observed in the large DNA viruses, such as the poxviruses, that can replicate in the cytoplasm. With a few exceptions, RNA viruses that infect animal cells replicate in the cytoplasm. An important exception that will be highlighted later is Influenza virus.

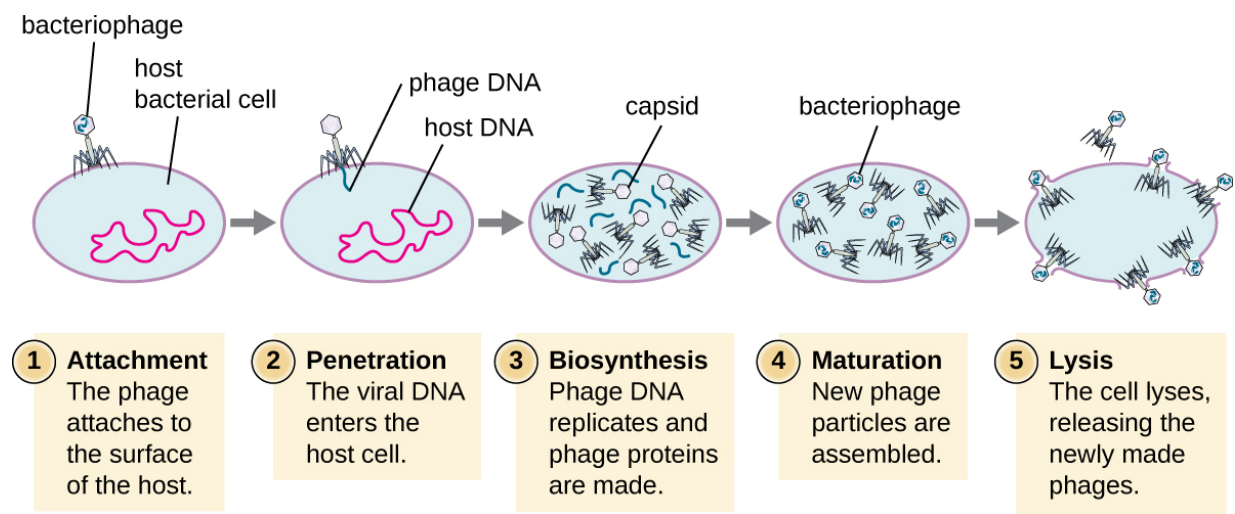
The Life Cycle of Viruses with Prokaryote Hosts

The life cycle of bacteriophages has been a good model for understanding how viruses affect the cells they infect, since similar processes have been observed for eukaryotic viruses, which can cause immediate death of the cell or establish a latent or chronic infection. **Virulent phages** typically lead

to the death of the cell through cell lysis. **Temperate phages**, on the other hand, can become part of a host chromosome and are replicated with the cell genome until such time as they are induced to make newly assembled viruses, or **progeny viruses**.

The Lytic Cycle

During the **lytic cycle** of virulent phage, the bacteriophage takes over the cell, reproduces new phages, and destroys the cell. T-even phage is a good example of a well-characterized class of virulent phages. There are five stages in the bacteriophage lytic cycle (see [\[link\]](#)). **Attachment** is the first stage in the infection process in which the phage interacts with specific bacterial surface receptors (e.g., lipopolysaccharides and OmpC protein on host surfaces). Most phages have a narrow host range and may infect one species of bacteria or one strain within a species. This unique recognition can be exploited for targeted treatment of bacterial infection by phage therapy or for phage typing to identify unique bacterial subspecies or strains. The second stage of infection is entry or **penetration**. This occurs through contraction of the tail sheath, which acts like a hypodermic needle to inject the viral genome through the cell wall and membrane. The phage head and remaining components remain outside the bacteria.



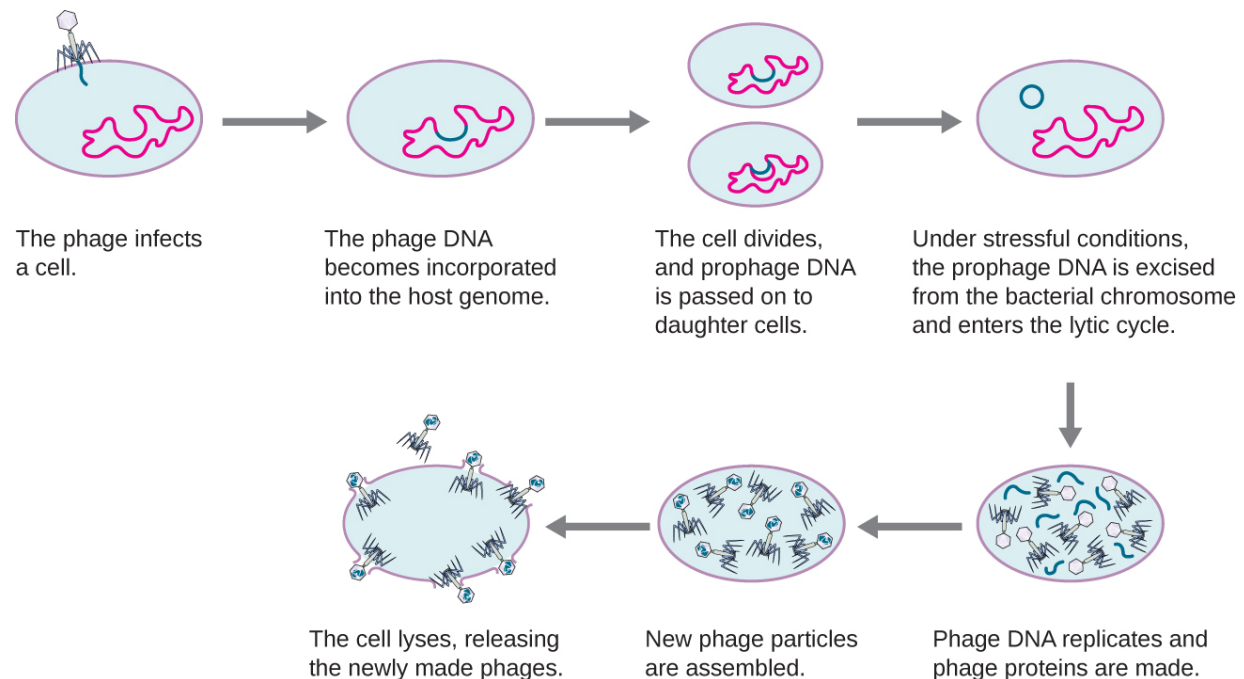
A virulent phage shows only the lytic cycle pictured here. In the lytic cycle, the phage replicates and lyses the host cell.

The third stage of infection is **biosynthesis** of new viral components. After entering the host cell, the virus synthesizes virus-encoded endonucleases to degrade the bacterial chromosome. It then hijacks the host cell to replicate, transcribe, and translate the necessary viral components (capsomeres, sheath, base plates, tail fibers, and viral enzymes) for the assembly of new viruses. Polymerase genes are usually expressed early in the cycle, while capsid and tail proteins are expressed later. During the **maturation** phase, new virions are created. To liberate free phages, the bacterial cell wall is disrupted by phage proteins such as holin or lysozyme. The final stage is release. Mature viruses burst out of the host cell in a process called **lysis** and the progeny viruses are liberated into the environment to infect new cells.

The Lysogenic Cycle

In a **lysogenic cycle**, the phage genome also enters the cell through attachment and penetration. A prime example of a phage with this type of life cycle is the lambda phage. During the lysogenic cycle, instead of killing the host, the phage genome integrates into the bacterial chromosome and becomes part of the host. The integrated phage genome is called a **prophage**. A bacterial host with a prophage is called a **lysogen**. The process in which a bacterium is infected by a temperate phage is called **lysogeny**. It is typical of temperate phages to be latent or inactive within the cell. As the bacterium replicates its chromosome, it also replicates the phage's DNA and passes it on to new daughter cells during reproduction. The presence of the phage may alter the phenotype of the bacterium, since it can bring in extra genes (e.g., toxin genes that can increase bacterial virulence). This change in the host phenotype is called **lysogenic conversion** or **phage conversion**. Some bacteria, such as *Vibrio cholerae* and *Clostridium botulinum*, are less virulent in the absence of the prophage. The phages infecting these bacteria carry the toxin genes in their genome and enhance

the virulence of the host when the toxin genes are expressed. In the case of *V. cholera*, phage encoded toxin can cause severe diarrhea; in *C. botulinum*, the toxin can cause paralysis. During lysogeny, the prophage will persist in the host chromosome until **induction**, which results in the excision of the viral genome from the host chromosome. After induction has occurred the temperate phage can proceed through a lytic cycle and then undergo lysogeny in a newly infected cell (see [\[link\]](#)).



A temperate bacteriophage has both lytic and lysogenic cycles. In the lysogenic cycle, phage DNA is incorporated into the host genome, forming a prophage, which is passed on to subsequent generations of cells. Environmental stressors such as starvation or exposure to toxic chemicals may cause the prophage to be excised and enter the lytic cycle.

Note:

This [video](#) illustrates the stages of the lysogenic life cycle of a bacteriophage and the transition to a lytic phase.

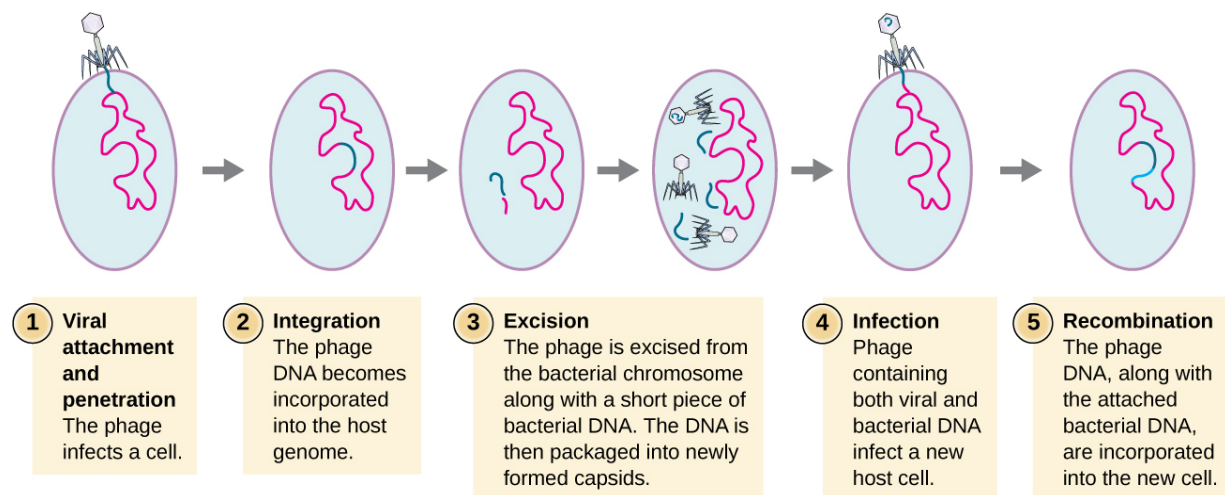
Note:

- Is a latent phage undetectable in a bacterium?

Transduction

Transduction occurs when a bacteriophage transfers bacterial DNA from one bacterium to another during sequential infections. There are two types of transduction: generalized and specialized transduction. During the lytic cycle of viral replication, the virus hijacks the host cell, degrades the host chromosome, and makes more viral genomes. As it assembles and packages DNA into the phage head, packaging occasionally makes a mistake. Instead of packaging viral DNA, it takes a random piece of host DNA and inserts it into the capsid. Once released, this virion will then inject the former host's DNA into a newly infected host. The asexual transfer of genetic information can allow for DNA recombination to occur, thus providing the new host with new genes (e.g., an antibiotic-resistance gene, or a sugar-metabolizing gene). **Generalized transduction** occurs when a random piece of bacterial chromosomal DNA is transferred by the phage during the lytic cycle. **Specialized transduction** occurs at the end of the lysogenic cycle, when the prophage is excised and the bacteriophage enters the lytic cycle. Since the phage is integrated into the host genome, the prophage can replicate as part of the host. However, some conditions (e.g., ultraviolet light exposure or chemical exposure) stimulate the prophage to undergo induction, causing the phage to excise from the genome, enter the lytic cycle, and produce new phages to leave host cells. During the process of excision from the host chromosome, a phage may occasionally remove some bacterial DNA near the site of viral integration. The phage and host DNA from one end or both ends of the integration site are packaged within the capsid and are transferred to the new, infected host. Since the DNA

transferred by the phage is not randomly packaged but is instead a specific piece of DNA near the site of integration, this mechanism of gene transfer is referred to as specialized transduction (see [\[link\]](#)). The DNA can then recombine with host chromosome, giving the latter new characteristics. Transduction seems to play an important role in the evolutionary process of bacteria, giving them a mechanism for asexual exchange of genetic information.



This flowchart illustrates the mechanism of specialized transduction.

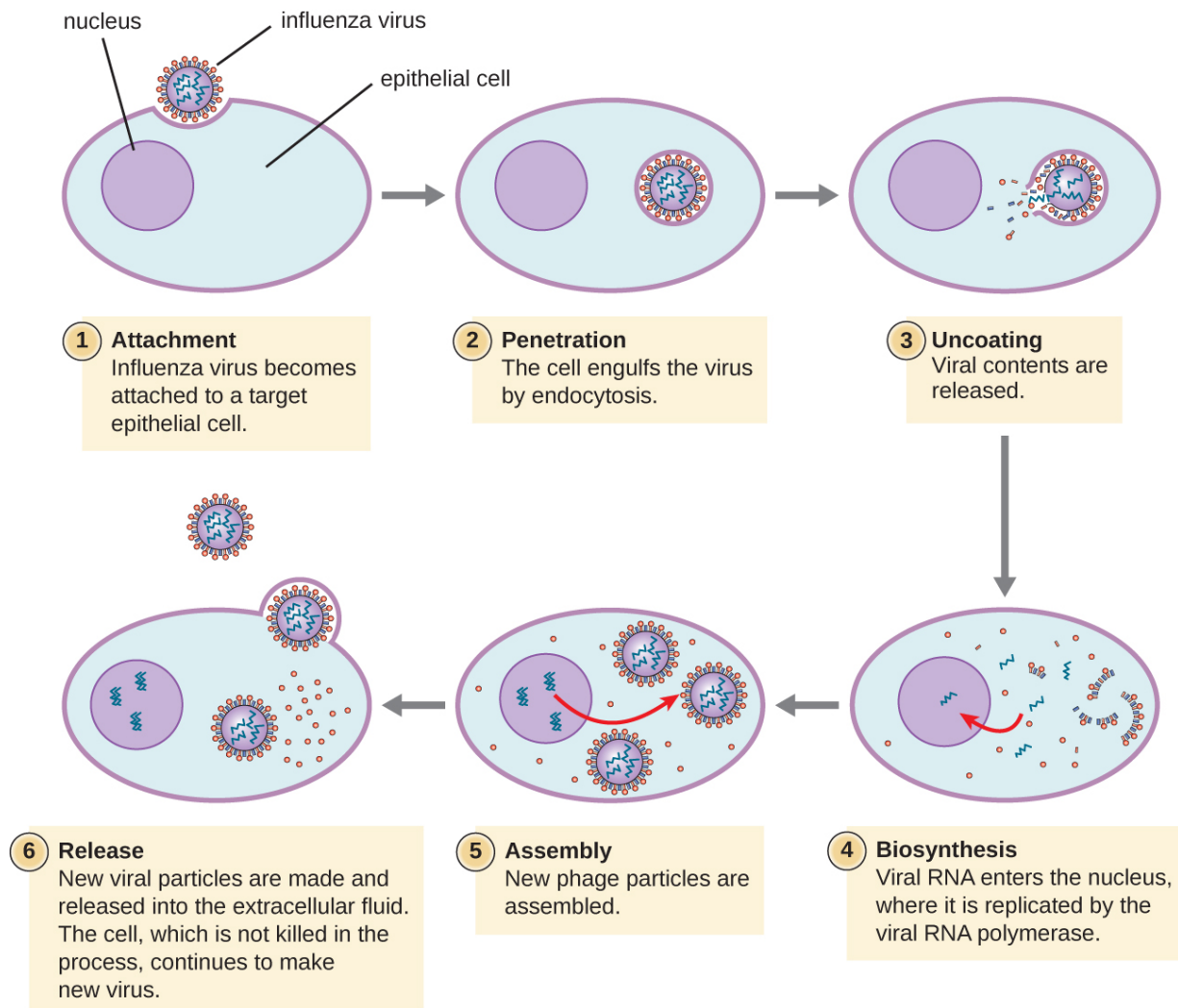
An integrated phage excises, bringing with it a piece of the DNA adjacent to its insertion point. On reinfection of a new bacterium, the phage DNA integrates along with the genetic material acquired from the previous host.

Note:

- Which phage life cycle is associated with which forms of transduction?

Life Cycle of Viruses with Animal Hosts

Lytic animal viruses follow similar infection stages to bacteriophages: attachment, penetration, biosynthesis, maturation, and release (see [\[link\]](#)). However, the mechanisms of penetration, nucleic-acid biosynthesis, and release differ between bacterial and animal viruses. After binding to host receptors, animal viruses enter through endocytosis (engulfment by the host cell) or through membrane fusion (viral envelope with the host cell membrane). Many viruses are host specific, meaning they only infect a certain type of host; and most viruses only infect certain types of cells within tissues. This specificity is called a **tissue tropism**. Examples of this are demonstrated by the poliovirus, which exhibits tropism for the tissues of the brain and spinal cord, or the influenza virus, which has a primary tropism for the respiratory tract.

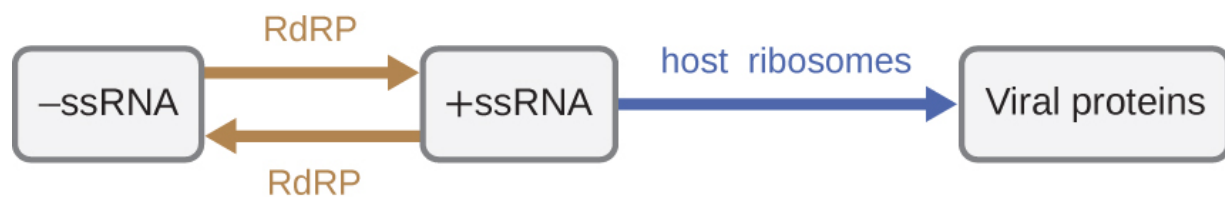


Influenza virus is one of the few RNA viruses that replicates in the nucleus of cells. In influenza virus infection, viral glycoproteins attach the virus to a host epithelial cell. As a result, the virus is engulfed. Viral RNA and viral proteins are made and assembled into new virions that are released by budding.

Animal viruses do not always express their genes using the normal flow of genetic information—from DNA to RNA to protein. Some viruses have a dsDNA genome like cellular organisms and can follow the normal flow. However, others may have ssDNA, dsRNA, or ssRNA genomes. The nature of the genome determines how the genome is replicated and expressed as

viral proteins. If a genome is ssDNA, host enzymes will be used to synthesize a second strand that is complementary to the genome strand, thus producing dsDNA. The dsDNA can now be replicated, transcribed, and translated similar to host DNA.

If the viral genome is RNA, a different mechanism must be used. There are three types of RNA genome: dsRNA, **positive (+) single-strand (+ssRNA)** or **negative (–) single-strand RNA (–ssRNA)**. If a virus has a +ssRNA genome, it can be translated directly to make viral proteins. Viral genomic +ssRNA acts like cellular mRNA. However, if a virus contains a –ssRNA genome, the host ribosomes cannot translate it until the –ssRNA is replicated into +ssRNA by viral RNA-dependent RNA polymerase (RdRP) (see [\[link\]](#)). The RdRP is brought in by the virus and can be used to make +ssRNA from the original –ssRNA genome. The RdRP is also an important enzyme for the replication of dsRNA viruses, because it uses the negative strand of the double-stranded genome as a template to create +ssRNA. The newly synthesized +ssRNA copies can then be translated by cellular ribosomes.



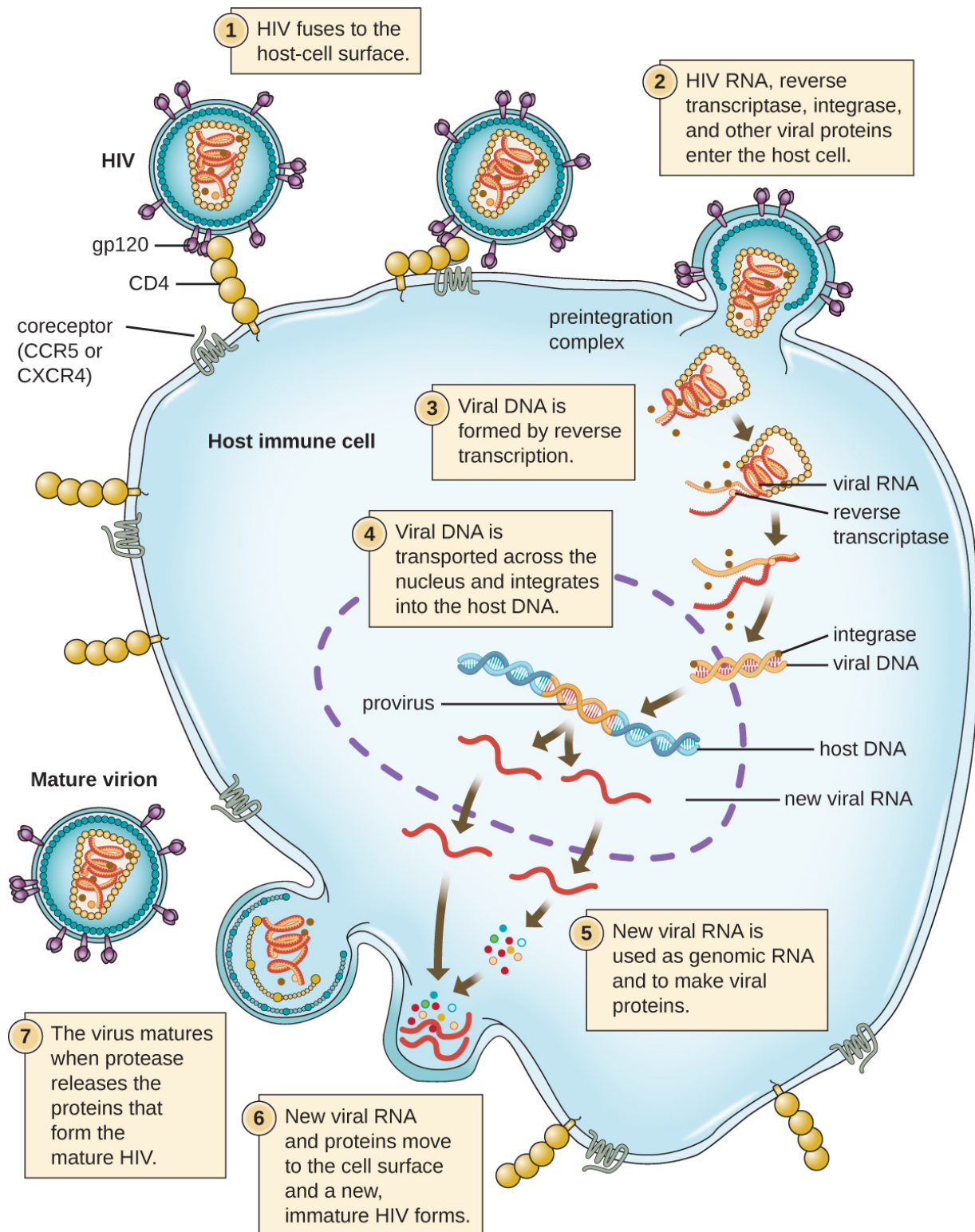
RdRP = viral RNA-dependent RNA polymerase

+ssRNA = positive (+) single strand

–ssRNA = negative (–) single-strand RNA

RNA viruses can contain +ssRNA that can be directly read by the ribosomes to synthesize viral proteins. Viruses containing –ssRNA must first use the –ssRNA as a template for the synthesis of +ssRNA before viral proteins can be synthesized.

An alternative mechanism for viral nucleic acid synthesis is observed in the **retroviruses**, which are +ssRNA viruses (see [\[link\]](#)). Single-stranded RNA viruses such as HIV carry a special enzyme called **reverse transcriptase** within the capsid that synthesizes a complementary ssDNA (cDNA) copy using the +ssRNA genome as a template. The ssDNA is then made into dsDNA, which can integrate into the host chromosome and become a permanent part of the host. The integrated viral genome is called a **provirus**. The virus now can remain in the host for a long time to establish a chronic infection. The provirus stage is similar to the prophage stage in a bacterial infection during the lysogenic cycle. However, unlike prophage, the provirus does not undergo excision after splicing into the genome.



HIV, an enveloped, icosahedral retrovirus, attaches to a cell surface receptor of an immune cell and fuses with the cell membrane. Viral contents are released into the cell, where viral enzymes convert the

single-stranded RNA genome into DNA and incorporate it into the host genome. (credit: modification of work by NIAID, NIH)

Note:

- Is RNA-dependent RNA polymerase made from a viral gene or a host gene?

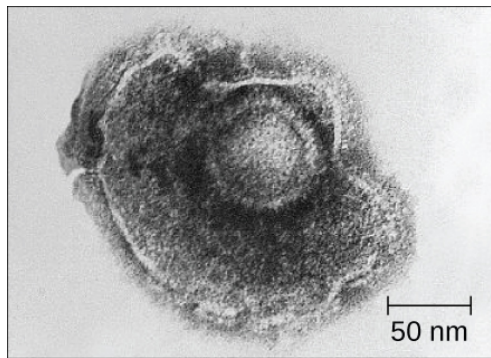
Persistent Infections

Persistent infection occurs when a virus is not completely cleared from the system of the host but stays in certain tissues or organs of the infected person. The virus may remain silent or undergo productive infection without seriously harming or killing the host. Mechanisms of persistent infection may involve the regulation of the viral or host gene expressions or the alteration of the host immune response. The two primary categories of persistent infections are latent infection and chronic infection. Examples of viruses that cause latent infections include herpes simplex virus (oral and genital herpes), varicella-zoster virus (chickenpox and shingles), and Epstein-Barr virus (mononucleosis). Hepatitis C virus and HIV are two examples of viruses that cause long-term chronic infections.

Latent Infection

Not all animal viruses undergo replication by the lytic cycle. There are viruses that are capable of remaining hidden or dormant inside the cell in a process called latency. These types of viruses are known as **latent viruses** and may cause latent infections. Viruses capable of latency may initially cause an acute infection before becoming dormant.

For example, the varicella-zoster virus infects many cells throughout the body and causes chickenpox, characterized by a rash of blisters covering the skin. About 10 to 12 days postinfection, the disease resolves and the virus goes dormant, living within nerve-cell ganglia for years. During this time, the virus does not kill the nerve cells or continue replicating. It is not clear why the virus stops replicating within the nerve cells and expresses few viral proteins but, in some cases, typically after many years of dormancy, the virus is reactivated and causes a new disease called shingles ([\[link\]](#)). Whereas chickenpox affects many areas throughout the body, shingles is a nerve cell-specific disease emerging from the ganglia in which the virus was dormant.



(a)



(b)

(a) Varicella-zoster, the virus that causes chickenpox, has an enveloped icosahedral capsid visible in this transmission electron micrograph. Its double-stranded DNA genome becomes incorporated in the host DNA.

(b) After a period of latency, the virus can reactivate in the form of shingles, usually manifesting as a painful, localized rash on one side of the body. (credit a: modification of work by Erskine Palmer and B.G.

Partin—scale-bar data from Matt Russell; credit b: modification of work by Rosmarie Voegtli)

Latent viruses may remain dormant by existing as circular viral genome molecules outside of the host chromosome. Others become proviruses by

integrating into the host genome. During dormancy, viruses do not cause any symptoms of disease and may be difficult to detect. A patient may be unaware that he or she is carrying the virus unless a viral diagnostic test has been performed.

Chronic Infection

A chronic infection is a disease with symptoms that are recurrent or persistent over a long time. Some viral infections can be chronic if the body is unable to eliminate the virus. HIV is an example of a virus that produces a chronic infection, often after a long period of latency. Once a person becomes infected with HIV, the virus can be detected in tissues continuously thereafter, but untreated patients often experience no symptoms for years. However, the virus maintains chronic persistence through several mechanisms that interfere with immune function, including preventing expression of viral antigens on the surface of infected cells, altering immune cells themselves, restricting expression of viral genes, and rapidly changing viral antigens through mutation. Eventually, the damage to the immune system results in progression of the disease leading to acquired immunodeficiency syndrome (AIDS). The various mechanisms that HIV uses to avoid being cleared by the immune system are also used by other chronically infecting viruses, including the hepatitis C virus.

Note:

- In what two ways can a virus manage to maintain a persistent infection?

Life Cycle of Viruses with Plant Hosts

Plant viruses are more similar to animal viruses than they are to bacteriophages. Plant viruses may be enveloped or non-enveloped. Like many animal viruses, plant viruses can have either a DNA or RNA genome and be single stranded or double stranded. However, most plant viruses do not have a DNA genome; the majority have a +ssRNA genome, which acts like messenger RNA (mRNA). Only a minority of plant viruses have other types of genomes.

Plant viruses may have a narrow or broad host range. For example, the citrus tristeza virus infects only a few plants of the *Citrus* genus, whereas the cucumber mosaic virus infects thousands of plants of various plant families. Most plant viruses are transmitted by contact between plants, or by fungi, nematodes, insects, or other arthropods that act as mechanical vectors. However, some viruses can only be transferred by a specific type of insect vector; for example, a particular virus might be transmitted by aphids but not whiteflies. In some cases, viruses may also enter healthy plants through wounds, as might occur due to pruning or weather damage.

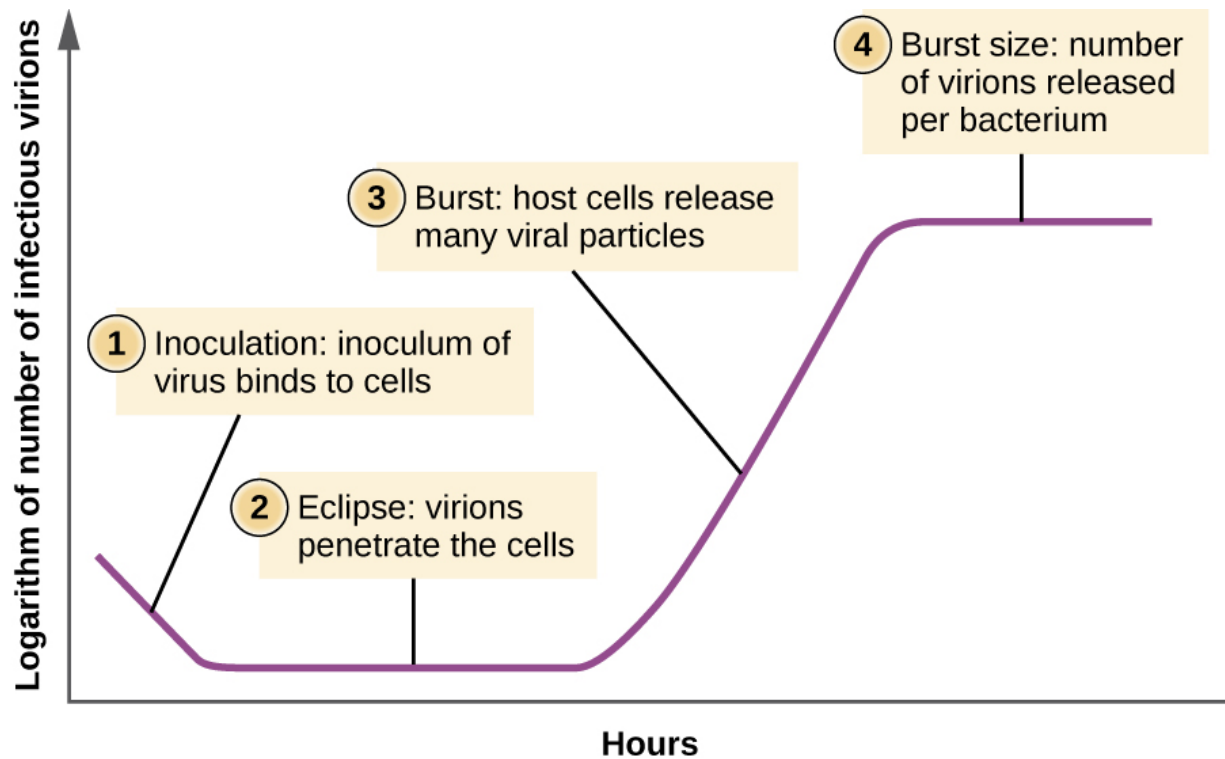
Viruses that infect plants are considered biotrophic parasites, which means that they can establish an infection without killing the host, similar to what is observed in the lysogenic life cycles of bacteriophages. Viral infection can be asymptomatic (latent) or can lead to cell death (lytic infection). The life cycle begins with the penetration of the virus into the host cell. Next, the virus is uncoated within the cytoplasm of the cell when the capsid is removed. Depending on the type of nucleic acid, cellular components are used to replicate the viral genome and synthesize viral proteins for assembly of new virions. To establish a systemic infection, the virus must enter a part of the vascular system of the plant, such as the phloem. The time required for systemic infection may vary from a few days to a few weeks depending on the virus, the plant species, and the environmental conditions. The virus life cycle is complete when it is transmitted from an infected plant to a healthy plant.

Note:

- What is the structure and genome of a typical plant virus?

Viral Growth Curve

Unlike the growth curve for a bacterial population, the growth curve for a virus population over its life cycle does not follow a sigmoidal curve. During the initial stage, an inoculum of virus causes infection. In the **eclipse phase**, viruses bind and penetrate the cells with no virions detected in the medium. The chief difference that next appears in the viral growth curve compared to a bacterial growth curve occurs when virions are released from the lysed host cell at the same time. Such an occurrence is called a **burst**, and the number of virions per bacterium released is described as the **burst size**. In a one-step multiplication curve for bacteriophage, the host cells lyse, releasing many viral particles to the medium, which leads to a very steep rise in **viral titer** (the number of virions per unit volume). If no viable host cells remain, the viral particles begin to degrade during the decline of the culture (see [\[link\]](#)).



The one-step multiplication curve for a bacteriophage population follows three steps: 1) inoculation, during which the virions attach to host cells; 2) eclipse, during which entry of the viral genome occurs; and 3) burst, when sufficient numbers of new virions are produced and emerge from the host cell. The burst size is the maximum number of virions produced per bacterium.

Note:

- What aspect of the life cycle of a virus leads to the sudden increase in the growth curve?

Note:

Unregistered Treatments

Ebola is incurable and deadly. The outbreak in West Africa in 2014 was unprecedented, dwarfing other human Ebola epidemics in the level of mortality. Of 24,666 suspected or confirmed cases reported, 10,179 people died.[\[footnote\]](#)

World Health Organization. “WHO Ebola Data and Statistics.” March 18, 2005. <http://apps.who.int/gho/data/view ebola-sitrep ebola-summary-20150318?lang=en>

No approved treatments or vaccines for Ebola are available. While some drugs have shown potential in laboratory studies and animal models, they have not been tested in humans for safety and effectiveness. Not only are these drugs untested or unregistered but they are also in short supply.

Given the great suffering and high mortality rates, it is fair to ask whether unregistered and untested medications are better than none at all. Should such drugs be dispensed and, if so, who should receive them, in light of their extremely limited supplies? Is it ethical to treat untested drugs on patients with Ebola? On the other hand, is it ethical to withhold potentially life-saving drugs from dying patients? Or should the drugs perhaps be reserved for health-care providers working to contain the disease?

In August 2014, two infected US aid workers and a Spanish priest were treated with ZMapp, an unregistered drug that had been tested in monkeys but not in humans. The two American aid workers recovered, but the priest died. Later that month, the WHO released a report on the ethics of treating patients with the drug. Since Ebola is often fatal, the panel reasoned that it is ethical to give the unregistered drugs and unethical to withhold them for safety concerns. This situation is an example of “compassionate use” outside the well-established system of regulation and governance of therapies.

Note:

Ebola in the US

On September 24, 2014, Thomas Eric Duncan arrived at the Texas Health Presbyterian Hospital in Dallas complaining of a fever, headache, vomiting, and diarrhea—symptoms commonly observed in patients with the cold or the flu. After examination, an emergency department doctor

diagnosed him with sinusitis, prescribed some antibiotics, and sent him home. Two days later, Duncan returned to the hospital by ambulance. His condition had deteriorated and additional blood tests confirmed that he has been infected with the Ebola virus.

Further investigations revealed that Duncan had just returned from Liberia, one of the countries in the midst of a severe Ebola epidemic. On September 15, nine days before he showed up at the hospital in Dallas, Duncan had helped transport an Ebola-stricken neighbor to a hospital in Liberia. The hospital continued to treat Duncan, but he died several days after being admitted.

The timeline of the Duncan case is indicative of the life cycle of the Ebola virus. The incubation time for Ebola ranges from 2 days to 21 days. Nine days passed between Duncan's exposure to the virus infection and the appearance of his symptoms. This corresponds, in part, to the eclipse period in the growth of the virus population. During the eclipse phase, Duncan would have been unable to transmit the disease to others.

However, once an infected individual begins exhibiting symptoms, the disease becomes very contagious. Ebola virus is transmitted through direct contact with droplets of bodily fluids such as saliva, blood, and vomit. Duncan could conceivably have transmitted the disease to others at any time after he began having symptoms, presumably some time before his arrival at the hospital in Dallas. Once a hospital realizes a patient like Duncan is infected with Ebola virus, the patient is immediately quarantined, and public health officials initiate a back trace to identify everyone with whom a patient like Duncan might have interacted during the period in which he was showing symptoms.

Public health officials were able to track down 10 high-risk individuals (family members of Duncan) and 50 low-risk individuals to monitor them for signs of infection. None contracted the disease. However, one of the nurses charged with Duncan's care did become infected. This, along with Duncan's initial misdiagnosis, made it clear that US hospitals needed to provide additional training to medical personnel to prevent a possible Ebola outbreak in the US.

- What types of training can prepare health professionals to contain emerging epidemics like the Ebola outbreak of 2014?

- What is the difference between a contagious pathogen and an infectious pathogen?



Researchers working with Ebola virus use layers of defenses against accidental infection, including protective clothing, breathing systems, and negative air-pressure cabinets for bench work. (credit: modification of work by Randal J. Schoepp)

Note:

For additional information about Ebola, please visit the [CDC](https://www.cdc.gov/ebola/) website.

Key Concepts and Summary

- Many viruses target specific hosts or tissues. Some may have more than one host.
- Many viruses follow several stages to infect host cells. These stages include **attachment, penetration, uncoating, biosynthesis, maturation, and release.**
- Bacteriophages have a **lytic** or **lysogenic cycle**. The lytic cycle leads to the death of the host, whereas the lysogenic cycle leads to integration of phage into the host genome.
- Bacteriophages inject DNA into the host cell, whereas animal viruses enter by endocytosis or membrane fusion.
- Animal viruses can undergo **latency**, similar to lysogeny for a bacteriophage.
- The majority of plant viruses are positive-strand ssRNA and can undergo latency, chronic, or lytic infection, as observed for animal viruses.
- The growth curve of bacteriophage populations is a **one-step multiplication curve** and not a sigmoidal curve, as compared to the bacterial growth curve.
- Bacteriophages transfer genetic information between hosts using either **generalized** or **specialized transduction.**

Multiple Choice

Exercise:

Problem:

Which of the following leads to the destruction of the host cells?

- A. lysogenic cycle
- B. lytic cycle
- C. prophage
- D. temperate phage

Solution:

B

Exercise:

Problem:

A virus obtains its envelope during which of the following phases?

- A. attachment
- B. penetration
- C. assembly
- D. release

Solution:

D

Exercise:

Problem:

Which of the following components is brought into a cell by HIV?

- A. a DNA-dependent DNA polymerase
- B. RNA polymerase
- C. ribosome
- D. reverse transcriptase

Solution:

D

Exercise:

Problem: A positive-strand RNA virus:

- A. must first be converted to a mRNA before it can be translated.
- B. can be used directly to translate viral proteins.
- C. will be degraded by host enzymes.
- D. is not recognized by host ribosomes.

Solution:

B

Exercise:

Problem:

What is the name for the transfer of genetic information from one bacterium to another bacterium by a phage?

- A. transduction
- B. penetration
- C. excision
- D. translation

Solution:

A

Fill in the Blank

Exercise:

Problem:

An enzyme from HIV that can make a copy of DNA from RNA is called _____.

Solution:

reverse transcriptase

Exercise:

Problem:

For lytic viruses, _____ is a phase during a viral growth curve when the virus is not detected.

Solution:

eclipse

Short Answer**Exercise:****Problem:**

Briefly explain the difference between the mechanism of entry of a T-even bacteriophage and an animal virus.

Exercise:**Problem:**

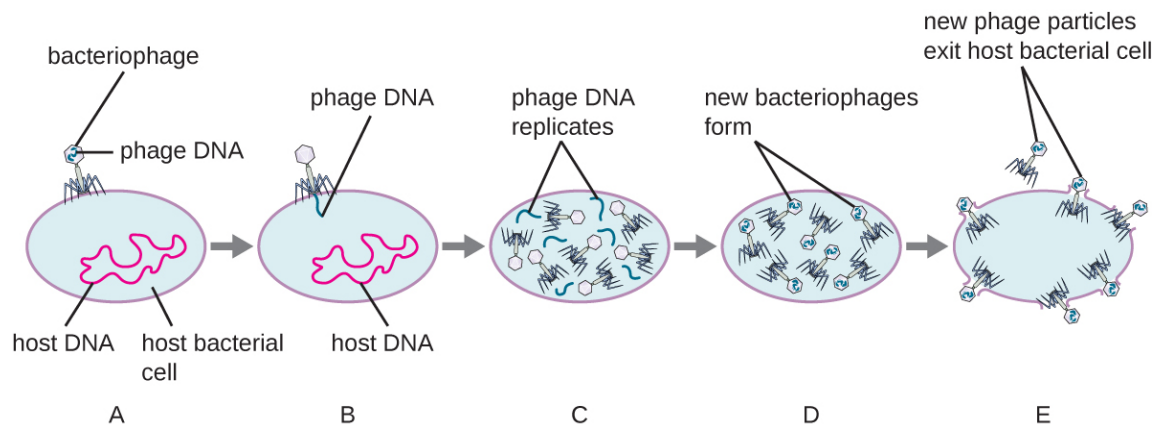
Discuss the difference between generalized and specialized transduction.

Exercise:

Problem: Differentiate between lytic and lysogenic cycles.

Critical Thinking**Exercise:****Problem:**

Label the five stages of a bacteriophage infection in the figure:



Exercise:

Problem:

Bacteriophages have lytic and lysogenic cycles. Discuss the advantages and disadvantages for the phage.

Exercise:

Problem:

How does reverse transcriptase aid a retrovirus in establishing a chronic infection?

Exercise:

Problem:

Discuss some methods by which plant viruses are transmitted from a diseased plant to a healthy one.

Isolation, Culture, and Identification of Viruses

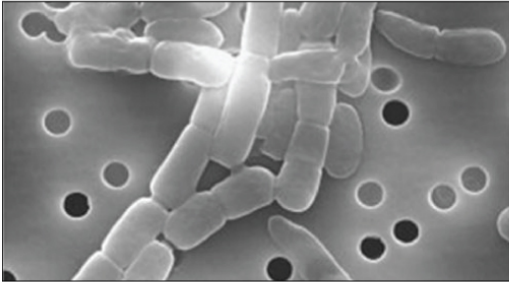
LEARNING OBJECTIVES

- Discuss why viruses were originally described as filterable agents
- Describe the cultivation of viruses and specimen collection and handling
- Compare in vivo and in vitro techniques used to cultivate viruses

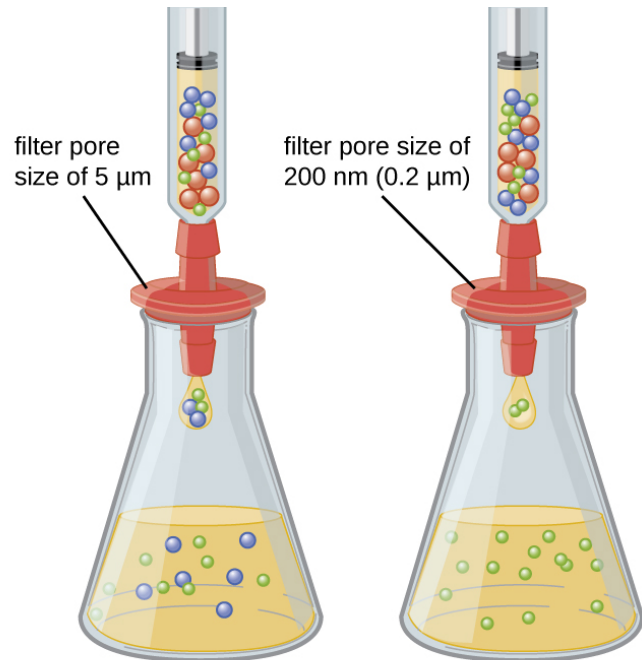
At the beginning of this chapter, we described how porcelain Chamberland filters with pores small enough to allow viruses to pass through were used to discover TMV. Today, porcelain filters have been replaced with membrane filters and other devices used to isolate and identify viruses.

Isolation of Viruses

Unlike bacteria, many of which can be grown on an artificial nutrient medium, viruses require a living host cell for replication. Infected host cells (eukaryotic or prokaryotic) can be cultured and grown, and then the growth medium can be harvested as a source of virus. Virions in the liquid medium can be separated from the host cells by either centrifugation or filtration. Filters can physically remove anything present in the solution that is larger than the virions; the viruses can then be collected in the filtrate (see [\[link\]](#)).



(a)



(b)

Membrane filters can be used to remove cells or viruses from a solution. (a) This scanning electron micrograph shows rod-shaped bacterial cells captured on the surface of a membrane filter. Note differences in the comparative size of the membrane pores and bacteria. Viruses will pass through this filter. (b) The size of the pores in the filter determines what is captured on the surface of the filter (animal [red] and bacteria [blue]) and removed from liquid passing through. Note the viruses (green) pass through the finer filter. (credit a: modification of work by U.S. Department of Energy)

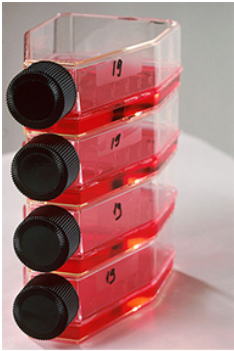
Note:

- What size filter pore is needed to collect a virus?

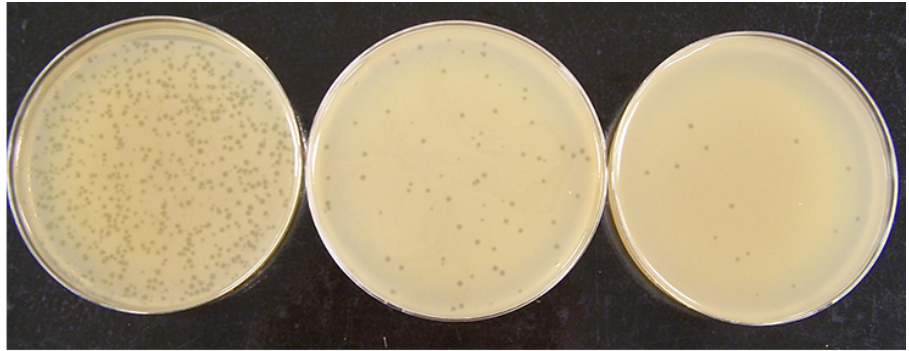
Cultivation of Viruses

Viruses can be grown **in vivo** (within a whole living organism, plant, or animal) or **in vitro** (outside a living organism in cells in an artificial environment, such as a test tube, cell culture flask, or agar plate).

Bacteriophages can be grown in the presence of a dense layer of bacteria (also called a **bacterial lawn**) grown in a 0.7 % soft agar in a Petri dish or flat (horizontal) flask (see [\[link\]](#)). The agar concentration is decreased from the 1.5% usually used in culturing bacteria. The soft 0.7% agar allows the bacteriophages to easily diffuse through the medium. For lytic bacteriophages, lysing of the bacterial hosts can then be readily observed when a clear zone called a **plaque** is detected (see [\[link\]](#)). As the phage kills the bacteria, many plaques are observed among the cloudy bacterial lawn.



(a)



(b)

(a) Flasks like this may be used to culture human or animal cells for viral culturing. (b) These plates contain bacteriophage T4 grown on an *Escherichia coli* lawn. Clear plaques are visible where host bacterial cells have been lysed. Viral titers increase on the plates to the left. (credit a: modification of work by National Institutes of Health; credit b: modification of work by American Society for Microbiology)

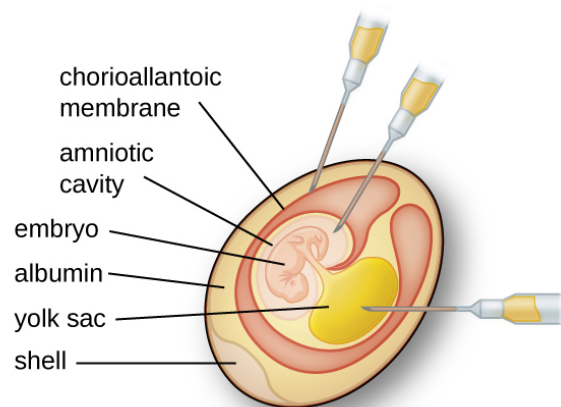
Animal viruses require cells within a host animal or tissue-culture cells derived from an animal. Animal virus cultivation is important for 1)

identification and diagnosis of pathogenic viruses in clinical specimens, 2) production of vaccines, and 3) basic research studies. In vivo host sources can be a developing embryo in an embryonated bird's egg (e.g., chicken, turkey) or a whole animal. For example, most of the influenza vaccine manufactured for annual flu vaccination programs is cultured in hens' eggs.

The embryo or host animal serves as an incubator for viral replication (see [\[link\]](#)). Location within the embryo or host animal is important. Many viruses have a tissue tropism, and must therefore be introduced into a specific site for growth. Within an embryo, target sites include the amniotic cavity, the chorioallantoic membrane, or the yolk sac. Viral infection may damage tissue membranes, producing lesions called pox; disrupt embryonic development; or cause the death of the embryo.



(a)

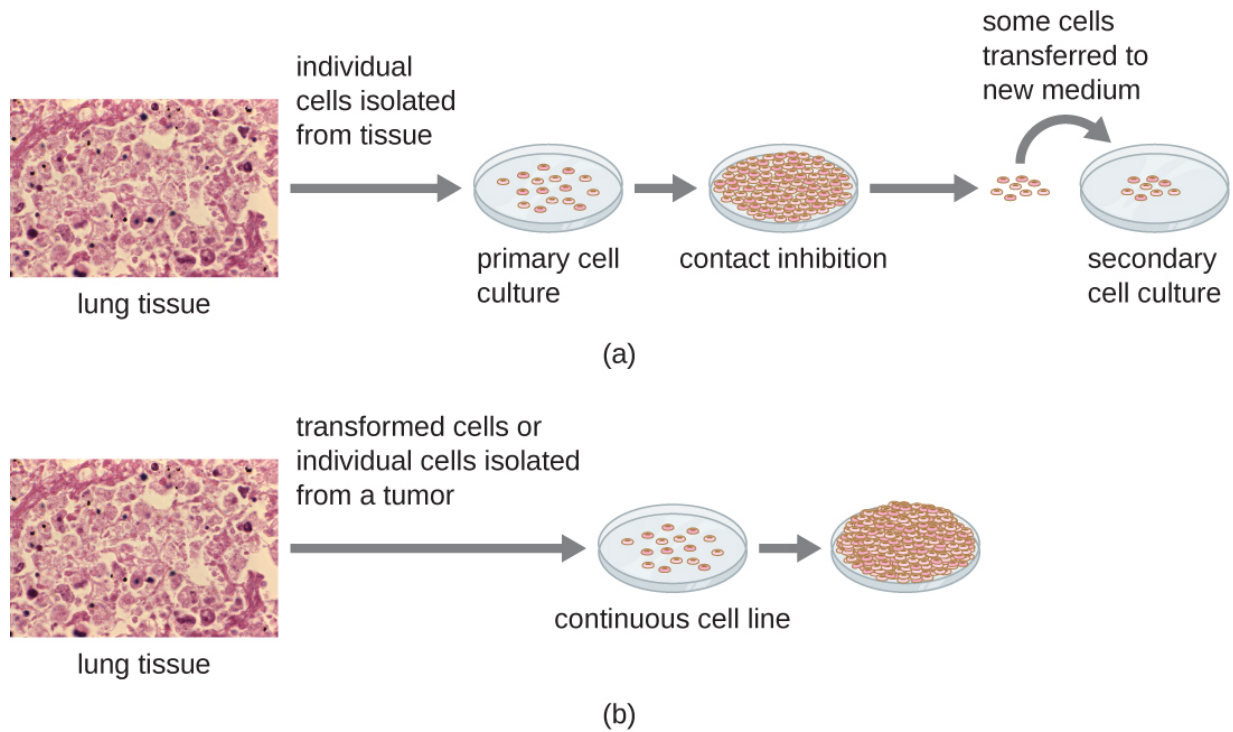


(b)

(a) The cells within chicken eggs are used to culture different types of viruses. (b) Viruses can be replicated in various locations within the egg, including the chorioallantoic membrane, the amniotic cavity, and the yolk sac. (credit a: modification of work by “Chung Hoang”/YouTube)

For in vitro studies, various types of cells can be used to support the growth of viruses. A primary cell culture is freshly prepared from animal organs or

tissues. Cells are extracted from tissues by mechanical scraping or mincing to release cells or by an enzymatic method using trypsin or collagenase to break up tissue and release single cells into suspension. Because of anchorage-dependence requirements, primary cell cultures require a liquid culture medium in a Petri dish or tissue-culture flask so cells have a solid surface such as glass or plastic for attachment and growth. Primary cultures usually have a limited life span. When cells in a primary culture undergo mitosis and a sufficient density of cells is produced, cells come in contact with other cells. When this cell-to-cell-contact occurs, mitosis is triggered to stop. This is called contact inhibition and it prevents the density of the cells from becoming too high. To prevent contact inhibition, cells from the primary cell culture must be transferred to another vessel with fresh growth medium. This is called a secondary cell culture. Periodically, cell density must be reduced by pouring off some cells and adding fresh medium to provide space and nutrients to maintain cell growth. In contrast to primary cell cultures, continuous cell lines, usually derived from transformed cells or tumors, are often able to be subcultured many times or even grown indefinitely (in which case they are called immortal). Continuous cell lines may not exhibit anchorage dependency (they will grow in suspension) and may have lost their contact inhibition. As a result, continuous cell lines can grow in piles or lumps resembling small tumor growths (see [\[link\]](#)).



Cells for culture are prepared by separating them from their tissue matrix. (a) Primary cell cultures grow attached to the surface of the culture container. Contact inhibition slows the growth of the cells once they become too dense and begin touching each other. At this point, growth can only be sustained by making a secondary culture. (b) Continuous cell cultures are not affected by contact inhibition. They continue to grow regardless of cell density. (credit “micrographs”: modification of work by Centers for Disease Control and Prevention)

An example of an immortal cell line is the HeLa cell line, which was originally cultivated from tumor cells obtained from Henrietta Lacks, a patient who died of cervical cancer in 1951. HeLa cells were the first continuous tissue-culture cell line and were used to establish tissue culture as an important technology for research in cell biology, virology, and medicine. Prior to the discovery of HeLa cells, scientists were not able to establish tissue cultures with any reliability or stability. More than six decades later, this cell line is still alive and being used for medical research. See [Eye on Ethics: The Immortal Cell Line of Henrietta Lacks](#) to read more

about this important cell line and the controversial means by which it was obtained.

Note:

- What property of cells makes periodic dilutions of primary cell cultures necessary?

Note:

The Immortal Cell Line of Henrietta Lacks

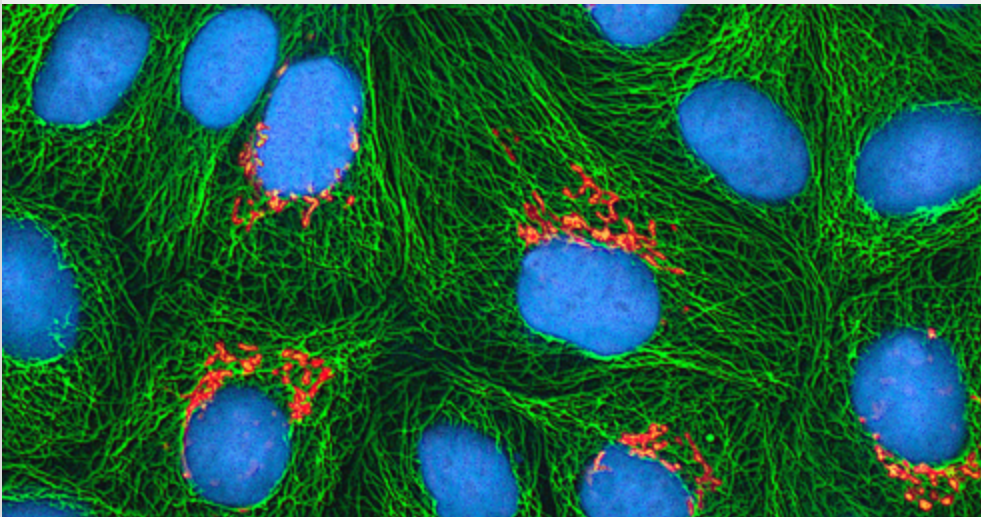
In January 1951, Henrietta Lacks, a 30-year-old African American woman from Baltimore, was diagnosed with cervical cancer at John Hopkins Hospital. We now know her cancer was caused by the human papillomavirus (HPV). Cytopathic effects of the virus altered the characteristics of her cells in a process called transformation, which gives the cells the ability to divide continuously. This ability, of course, resulted in a cancerous tumor that eventually killed Mrs. Lacks in October at age 31. Before her death, samples of her cancerous cells were taken without her knowledge or permission. The samples eventually ended up in the possession of Dr. George Gey, a biomedical researcher at Johns Hopkins University. Gey was able to grow some of the cells from Lacks's sample, creating what is known today as the immortal HeLa cell line. These cells have the ability to live and grow indefinitely and, even today, are still widely used in many areas of research.

According to Lacks's husband, neither Henrietta nor the family gave the hospital permission to collect her tissue specimen. Indeed, the family was not aware until 20 years after Lacks's death that her cells were still alive and actively being used for commercial and research purposes. Yet HeLa cells have been pivotal in numerous research discoveries related to polio, cancer, and AIDS, among other diseases. The cells have also been commercialized, although they have never themselves been patented. Despite this, Henrietta Lacks's estate has never benefited from the use of

the cells, although, in 2013, the Lacks family was given control over the publication of the genetic sequence of her cells.

This case raises several bioethical issues surrounding patients' informed consent and the right to know. At the time Lacks's tissues were taken, there were no laws or guidelines about informed consent. Does that mean she was treated fairly at the time? Certainly by today's standards, the answer would be no. Harvesting tissue or organs from a dying patient without consent is not only considered unethical but illegal, regardless of whether such an act could save other patients' lives. Is it ethical, then, for scientists to continue to use Lacks's tissues for research, even though they were obtained illegally by today's standards?

Ethical or not, Lacks's cells are widely used today for so many applications that it is impossible to list them all. Is this a case in which the ends justify the means? Would Lacks be pleased to know about her contribution to science and the millions of people who have benefited? Would she want her family to be compensated for the commercial products that have been developed using her cells? Or would she feel violated and exploited by the researchers who took part of her body without her consent? Because she was never asked, we will never know.

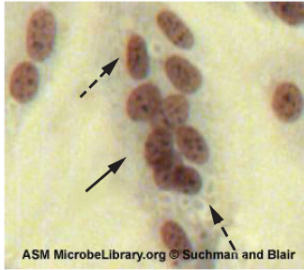
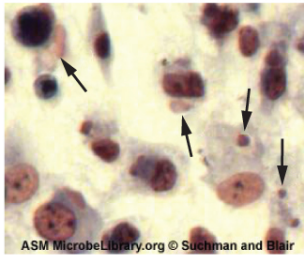
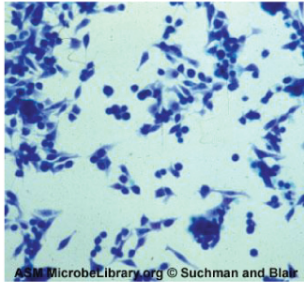


A multiphoton fluorescence image of HeLa cells in culture. Various fluorescent stains have been used to show the DNA (cyan), microtubules (green), and Golgi apparatus (orange). (credit: modification of work by National Institutes of Health)

Detection of a Virus

Regardless of the method of cultivation, once a virus has been introduced into a whole host organism, embryo, or tissue-culture cell, a sample can be prepared from the infected host, embryo, or cell line for further analysis under a brightfield, electron, or fluorescent microscope. **Cytopathic effects (CPEs)** are distinct observable cell abnormalities due to viral infection. CPEs can include loss of adherence to the surface of the container, changes in cell shape from flat to round, shrinkage of the nucleus, vacuoles in the cytoplasm, fusion of cytoplasmic membranes and the formation of multinucleated syncytia, inclusion bodies in the nucleus or cytoplasm, and complete cell lysis (see [link](#)).

Further pathological changes include viral disruption of the host genome and altering normal cells into transformed cells, which are the types of cells associated with carcinomas and sarcomas. The type or severity of the CPE depends on the type of virus involved. [link](#) lists CPEs for specific viruses.

Cytopathic Effects of Specific Viruses		
Virus	Cytopathic Effect	Example
<i>Paramyxovirus</i>	Syncytium and faint basophilic cytoplasmic inclusion bodies	 A light micrograph showing a syncytium, a multinucleated cell formed by the fusion of infected cells. Several dark, basophilic cytoplasmic inclusion bodies are visible. A solid arrow points to one inclusion body, and a dashed arrow points to another. The text 'ASM MicrobeLibrary.org © Suchman and Blair' is at the bottom.
<i>Poxvirus</i>	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	 A light micrograph showing several cells with prominent, pink, eosinophilic cytoplasmic inclusion bodies. Several black arrows point to these inclusion bodies. The cells appear slightly swollen. The text 'ASM MicrobeLibrary.org © Suchman and Blair' is at the bottom.
<i>Herpesvirus</i>	Cytoplasmic stranding (arrow) and nuclear inclusion bodies (dashed arrow)	 A light micrograph showing cells with cytoplasmic stranding, indicated by a solid black arrow. A dashed black arrow points to a nuclear inclusion body. The text 'ASM MicrobeLibrary.org © Suchman and Blair' is at the bottom.
<i>Adenovirus</i>	Cell enlargement, rounding, and distinctive "grape-like" clusters	 A light micrograph showing a cluster of cells that are enlarged and rounded, characteristic of adenovirus infection. The cells are stained blue. The text 'ASM MicrobeLibrary.org © Suchman and Blair' is at the bottom.

(credit "micrographs": modification of work by American Society for Microbiology)

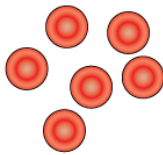
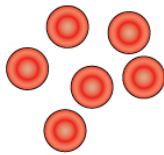
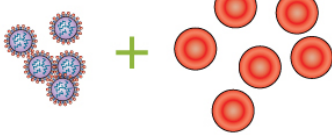
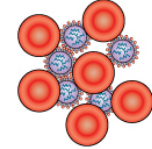
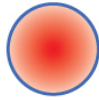
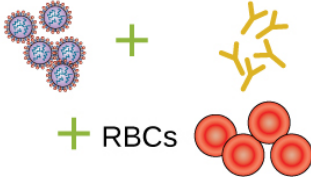
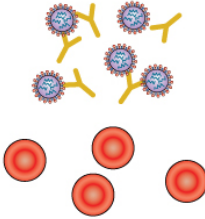
Note:

Watch this [video](#) to learn about the effects of viruses on cells.

Hemagglutination Assay

A serological assay is used to detect the presence of certain types of viruses in patient serum. Serum is the straw-colored liquid fraction of blood plasma from which clotting factors have been removed. Serum can be used in a direct assay called a hemagglutination assay to detect specific types of viruses in the patient's sample. Hemagglutination is the agglutination (clumping) together of erythrocytes (red blood cells). Many viruses produce surface proteins or spikes called hemagglutinins that can bind to receptors on the membranes of erythrocytes and cause the cells to agglutinate. Hemagglutination is observable without using the microscope, but this method does not always differentiate between infectious and noninfectious viral particles, since both can agglutinate erythrocytes.

To identify a specific pathogenic virus using hemagglutination, we must use an indirect approach. Proteins called antibodies, generated by the patient's immune system to fight a specific virus, can be used to bind to components such as hemagglutinins that are uniquely associated with specific types of viruses. The binding of the antibodies with the hemagglutinins found on the virus subsequently prevent erythrocytes from directly interacting with the virus. So when erythrocytes are added to the antibody-coated viruses, there is no appearance of agglutination; agglutination has been inhibited. We call these types of indirect assays for virus-specific antibodies hemagglutination inhibition (HAI) assays. HAI can be used to detect the presence of antibodies specific to many types of viruses that may be causing or have caused an infection in a patient even months or years after infection (see [\[link\]](#)). This assay is described in greater detail in [Agglutination Assays](#).

	Components	Interaction	Microtiter Results
A	RBCs 		No reaction 
B	Virus + RBCs 		Hemagglutination 
C	Virus + Antibody + RBCs 		Hemagglutination inhibition 

This chart shows the possible outcomes of a hemagglutination test. Row A: Erythrocytes do not bind together and will sink to the bottom of the well plate; this becomes visible as a red dot in the center of the well. Row B: Many viruses have hemagglutinins that causes agglutination of erythrocytes; the resulting hemagglutination forms a lattice structure that results in red color throughout the well. Row C: Virus-specific antibody, the viruses, and the erythrocytes are added to the well plate. The virus-specific antibodies inhibit agglutination, as can be seen as a red dot in the bottom of the well. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- What is the outcome of a positive HIA test?

Nucleic Acid Amplification Test

Nucleic acid amplification tests (NAAT) are used in molecular biology to detect unique nucleic acid sequences of viruses in patient samples.

Polymerase chain reaction (PCR) is an NAAT used to detect the presence of viral DNA in a patient's tissue or body fluid sample. PCR is a technique that amplifies (i.e., synthesizes many copies) of a viral DNA segment of interest. Using PCR, short nucleotide sequences called primers bind to specific sequences of viral DNA, enabling identification of the virus.

Reverse transcriptase-PCR (RT-PCR) is an NAAT used to detect the presence of RNA viruses. RT-PCR differs from PCR in that the enzyme reverse transcriptase (RT) is used to make a cDNA from the small amount of viral RNA in the specimen. The cDNA can then be amplified by PCR. Both PCR and RT-PCR are used to detect and confirm the presence of the viral nucleic acid in patient specimens.

Note:

HPV Scare

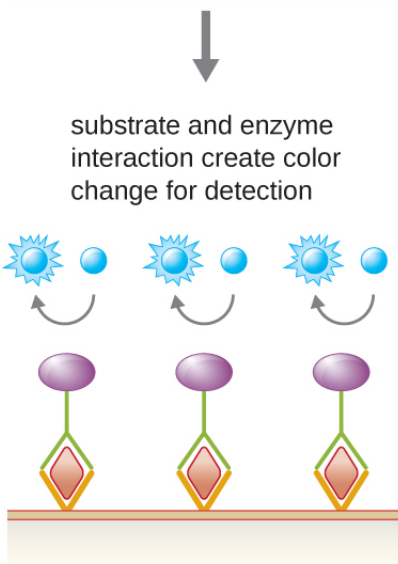
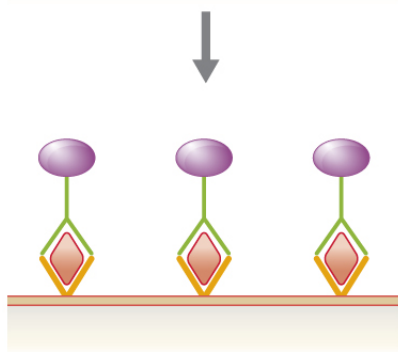
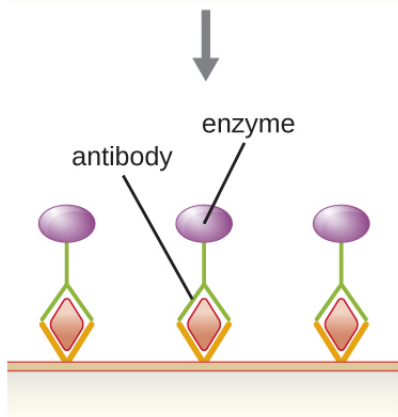
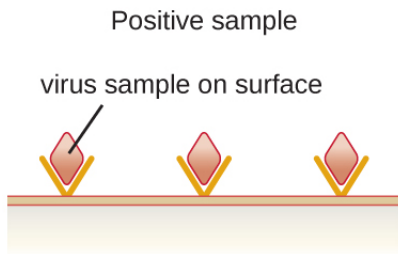
Michelle, a 21-year-old nursing student, came to the university clinic worried that she might have been exposed to a sexually transmitted disease (STD). Her sexual partner had recently developed several bumps on the base of his penis. He had put off going to the doctor, but Michelle suspects they are genital warts caused by HPV. She is especially concerned because she knows that HPV not only causes warts but is a prominent cause of cervical cancer. She and her partner always use condoms for contraception, but she is not confident that this precaution will protect her from HPV. Michelle's physician finds no physical signs of genital warts or any other STDs, but recommends that Michelle get a Pap smear along with an HPV test. The Pap smear will screen for abnormal cervical cells and the CPEs associated with HPV; the HPV test will test for the presence of the virus. If both tests are negative, Michelle can be more assured that she most likely has not become infected with HPV. However, her doctor suggests it might

be wise for Michelle to get vaccinated against HPV to protect herself from possible future exposure.

- Why does Michelle's physician order two different tests instead of relying on one or the other?

Enzyme Immunoassay

Enzyme immunoassays (EIAs) rely on the ability of antibodies to detect and attach to specific biomolecules called antigens. The detecting antibody attaches to the target antigen with a high degree of specificity in what might be a complex mixture of biomolecules. Also included in this type of assay is a colorless enzyme attached to the detecting antibody. The enzyme acts as a tag on the detecting antibody and can interact with a colorless substrate, leading to the production of a colored end product. EIAs often rely on layers of antibodies to capture and react with antigens, all of which are attached to a membrane filter (see [\[link\]](#)). EIAs for viral antigens are often used as preliminary screening tests. If the results are positive, further confirmation will require tests with even greater sensitivity, such as a western blot or an NAAT. EIAs are discussed in more detail in [EIAs and ELISAs](#).

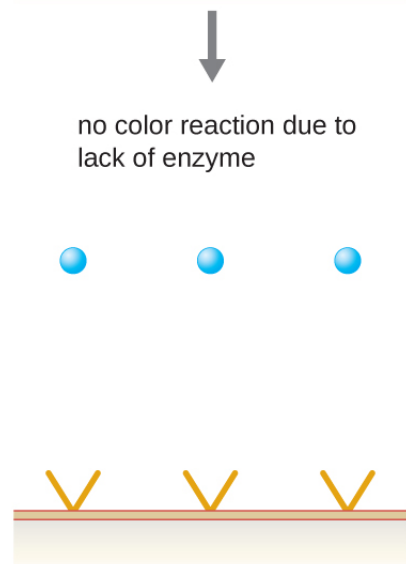
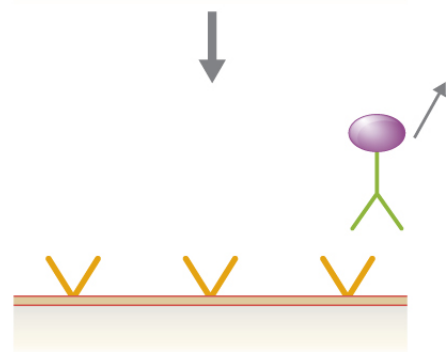
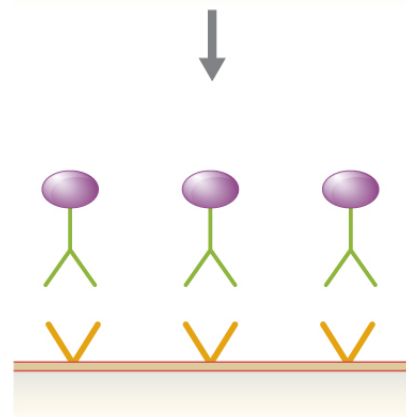
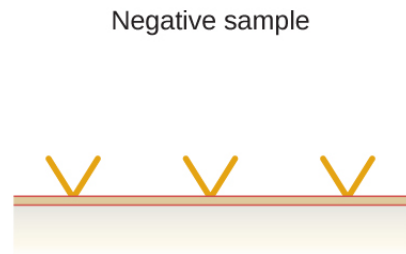


1 Apply patient sample to membrane filter.

2 Add antibody with enzyme conjugate. Antibody attaches to antigen if present.

3 Wash to remove unattached conjugate.

4 Add substrate.



Similar to rapid, over-the-counter pregnancy tests, EIAs for viral

antigens require a few drops of diluted patient serum or plasma applied to a membrane filter. The membrane filter has been previously modified and embedded with antibody to viral antigen and internal controls. Antibody conjugate is added to the filter, with the targeted antibody attached to the antigen (in the case of a positive test). Excess conjugate is washed off the filter. Substrate is added to activate the enzyme-mediated reaction to reveal the color change of a positive test. (credit: modification of work by “Cavetri”/Wikimedia Commons)

Note:

- What typically indicates a positive EIA test?

Note:

Part 3

Along with the RT/PCR analysis, David’s saliva was also collected for viral cultivation. In general, no single diagnostic test is sufficient for antemortem diagnosis, since the results will depend on the sensitivity of the assay, the quantity of virions present at the time of testing, and the timing of the assay, since release of virions in the saliva can vary. As it turns out, the result was negative for viral cultivation from the saliva. This is not surprising to David’s doctor, because one negative result is not an absolute indication of the absence of infection. It may be that the number of virions in the saliva is low at the time of sampling. It is not unusual to repeat the test at intervals to enhance the chance of detecting higher virus loads.

- Should David’s doctor modify his course of treatment based on these test results?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Viral cultivation requires the presence of some form of host cell (whole organism, embryo, or cell culture).
- Viruses can be isolated from samples by filtration.
- Viral filtrate is a rich source of released virions.
- Bacteriophages are detected by presence of clear **plaques** on bacterial lawn.
- Animal and plant viruses are detected by **cytopathic effects**, molecular techniques (PCR, RT-PCR), enzyme immunoassays, and serological assays (hemagglutination assay, hemagglutination inhibition assay).

Multiple Choice

Exercise:

Problem: Which of the followings cannot be used to culture viruses?

- A. tissue culture
- B. liquid medium only
- C. embryo
- D. animal host

Solution:

B

Exercise:

Problem:

Which of the following tests can be used to detect the presence of a specific virus?

- A. EIA
- B. RT-PCR
- C. PCR
- D. all of the above

Solution:

D

Exercise:

Problem: Which of the following is NOT a cytopathic effect?

- A. transformation
- B. cell fusion
- C. mononucleated cell
- D. inclusion bodies

Solution:

C

Fill in the Blank**Exercise:****Problem:**

Viruses can be diagnosed and observed using a(n) _____ microscope.

Solution:

Electron

Exercise:**Problem:**

Cell abnormalities resulting from a viral infection are called
_____.

Solution:

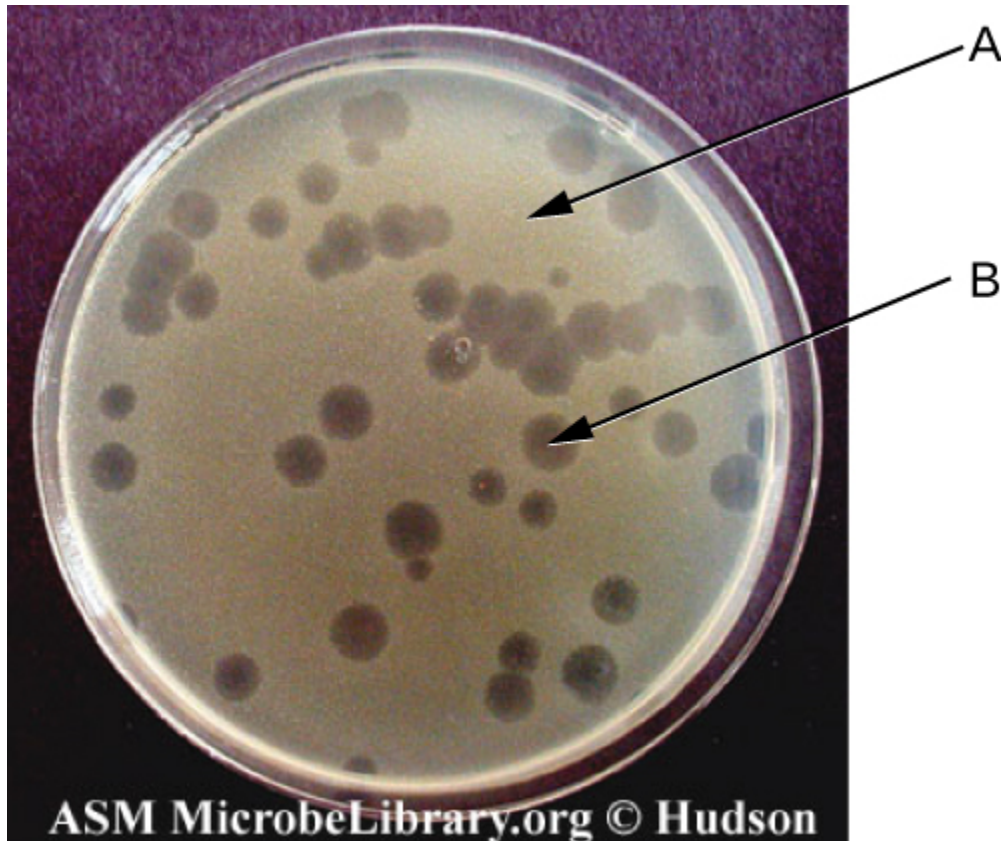
cytopathic effects

Short Answer**Exercise:**

Problem: Briefly explain the various methods of culturing viruses.

Critical Thinking**Exercise:**

Problem: Label the components indicated by arrows.



(credit: modification of work by American Society for Microbiology)

Exercise:

Problem:

What are some characteristics of the viruses that are similar to a computer virus?

Viroids, Virusoids, and Prions

LEARNING OBJECTIVES

- Describe viroids and their unique characteristics
- Describe virusoids and their unique characteristics
- Describe prions and their unique characteristics

Research attempts to discover the causative agents of previously uninvestigated diseases have led to the discovery of nonliving disease agents quite different from viruses. These include particles consisting only of RNA or only of protein that, nonetheless, are able to self-propagate at the expense of a host—a key similarity to viruses that allows them to cause disease conditions. To date, these discoveries include viroids, virusoids, and the proteinaceous prions.

Viroids

In 1971, Theodor Diener, a pathologist working at the Agriculture Research Service, discovered an acellular particle that he named a viroid, meaning “virus-like.” **Viroids** consist only of a short strand of circular RNA capable of self-replication. The first viroid discovered was found to cause potato tuber spindle disease, which causes slower sprouting and various deformities in potato plants (see [\[link\]](#)). Like viruses, potato spindle tuber viroids (PSTVs) take control of the host machinery to replicate their RNA genome. Unlike viruses, viroids do not have a protein coat to protect their genetic information.



These potatoes have been infected by the potato spindle tuber viroid (PSTV), which is typically spread when infected knives are used to cut healthy potatoes, which are then planted. (credit: Pamela Roberts, University of Florida Institute of Food and Agricultural Sciences, USDA ARS)

Viroids can result in devastating losses of commercially important agricultural food crops grown in fields and orchards. Since the discovery of PSTV, other viroids have been discovered that cause diseases in plants. Tomato planta macho viroid (TPMVd) infects tomato plants, which causes loss of chlorophyll, disfigured and brittle leaves, and very small tomatoes, resulting in loss of productivity in this field crop. Avocado sunblotch viroid (ASBVd) results in lower yields and poorer-quality fruit. ASBVd is the smallest viroid discovered thus far that infects plants. Peach latent mosaic viroid (PLMVd) can cause necrosis of flower buds and branches, and wounding of ripened fruit, which leads to fungal and bacterial growth in the fruit. PLMVd can also cause similar pathological changes in plums, nectarines, apricots, and cherries, resulting in decreased productivity in these orchards, as well. Viroids, in general, can be dispersed mechanically during crop maintenance or harvesting, vegetative

reproduction, and possibly via seeds and insects, resulting in a severe drop in food availability and devastating economic consequences.

Note:

- What is the genome of a viroid made of?

Virusoids

A second type of pathogenic RNA that can infect commercially important agricultural crops are the **virusoids**, which are subviral particles best described as non–self-replicating ssRNAs. RNA replication of virusoids is similar to that of viroids but, unlike viroids, virusoids require that the cell also be infected with a specific “helper” virus. There are currently only five described types of virusoids and their associated helper viruses. The helper viruses are all from the family of Sobemoviruses. An example of a helper virus is the subterranean clover mottle virus, which has an associated virusoid packaged inside the viral capsid. Once the helper virus enters the host cell, the virusoids are released and can be found free in plant cell cytoplasm, where they possess ribozyme activity. The helper virus undergoes typical viral replication independent of the activity of the virusoid. The virusoid genomes are small, only 220 to 388 nucleotides long. A virusoid genome does not code for any proteins, but instead serves only to replicate virusoid RNA.

Virusoids belong to a larger group of infectious agents called satellite RNAs, which are similar pathogenic RNAs found in animals. Unlike the plant virusoids, satellite RNAs may encode for proteins; however, like plant virusoids, satellite RNAs must coinfect with a helper virus to replicate. One satellite RNA that infects humans and that has been described by some scientists as a virusoid is the hepatitis delta virus (HDV), which, by some reports, is also called hepatitis delta virusoid. Much larger than a plant virusoid, HDV has a circular, ssRNA genome of 1,700 nucleotides and can direct the biosynthesis of HDV-associated proteins. The HDV helper virus is the hepatitis B virus (HBV). Coinfection with HBV and HDV results in more severe

pathological changes in the liver during infection, which is how HDV was first discovered.

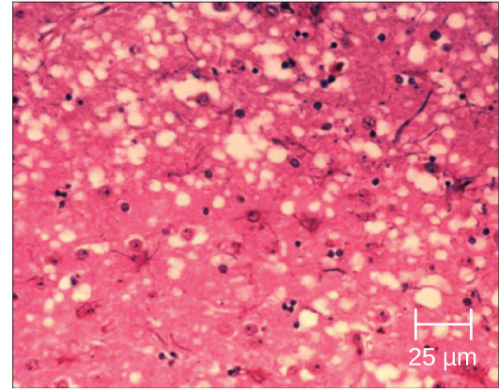
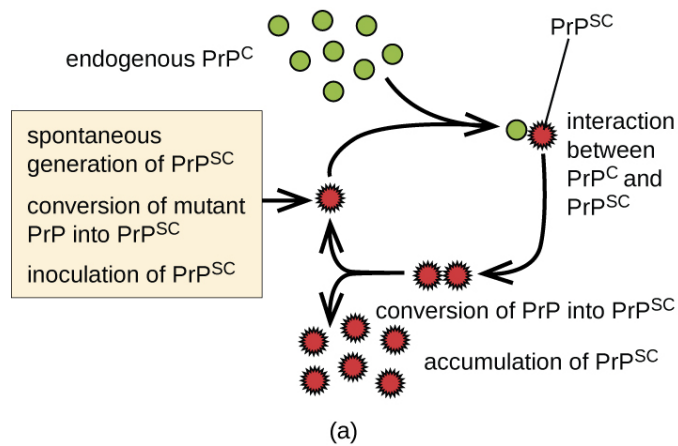
Note:

- What is the main difference between a viroid and a virusoid?

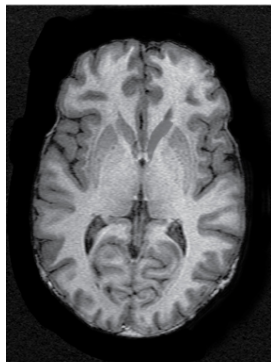
Prions

At one time, scientists believed that any infectious particle must contain DNA or RNA. Then, in 1982, Stanley Prusiner, a medical doctor studying scrapie (a fatal, degenerative disease in sheep) discovered that the disease was caused by proteinaceous infectious particles, or **prions**. Because proteins are acellular and do not contain DNA or RNA, Prusiner's findings were originally met with resistance and skepticism; however, his research was eventually validated, and he received the Nobel Prize in Physiology or Medicine in 1997.

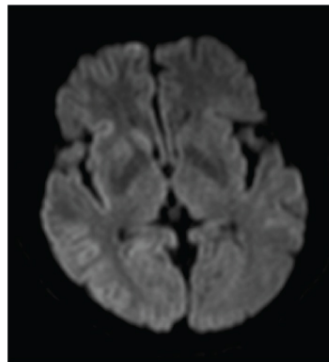
A prion is a misfolded rogue form of a normal protein (PrP^c) found in the cell. This rogue prion protein (PrP^{sc}), which may be caused by a genetic mutation or occur spontaneously, can be infectious, stimulating other endogenous normal proteins to become misfolded, forming plaques (see [\[link\]](#)). Today, prions are known to cause various forms of **transmissible spongiform encephalopathy** (TSE) in human and animals. TSE is a rare degenerative disorder that affects the brain and nervous system. The accumulation of rogue proteins causes the brain tissue to become sponge-like, killing brain cells and forming holes in the tissue, leading to brain damage, loss of motor coordination, and dementia (see [\[link\]](#)). Infected individuals are mentally impaired and become unable to move or speak. There is no cure, and the disease progresses rapidly, eventually leading to death within a few months or years.



Endogenous normal prion protein (PrP^C) is converted into the disease-causing form (PrP^{Sc}) when it encounters this variant form of the protein. PrP^{Sc} may arise spontaneously in brain tissue, especially if a mutant form of the protein is present, or it may originate from misfolded prions consumed in food that eventually find their way into brain tissue. (credit b: modification of work by USDA)

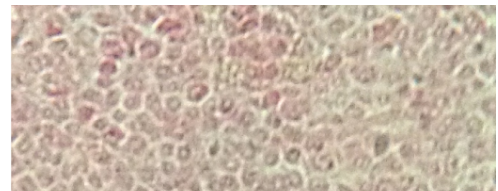


Normal brain

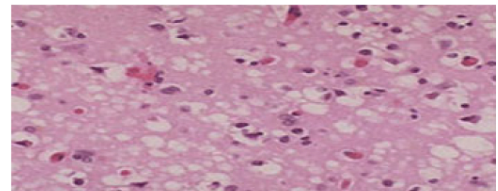


CJD brain

(a)



Normal brain tissue



Sponge-like lesions in the brain tissue of a CJD patient

(b)

Creutzfeldt-Jakob disease (CJD) is a fatal disease that causes degeneration of neural tissue. (a) These brain scans compare a normal brain to one with

CJD. (b) Compared to a normal brain, the brain tissue of a CJD patient is full of sponge-like lesions, which result from abnormal formations of prion protein. (credit a (right): modification of work by Dr. Laughlin Dawes; credit b (top): modification of work by Suzanne Wakim; credit b (bottom): modification of work by Centers for Disease Control and Prevention)

TSEs in humans include kuru, fatal familial insomnia, Gerstmann-Straussler-Scheinker disease, and Creutzfeldt-Jakob disease (see [\[link\]](#)). TSEs in animals include mad cow disease, scrapie (in sheep and goats), and chronic wasting disease (in elk and deer). TSEs can be transmitted between animals and from animals to humans by eating contaminated meat or animal feed. Transmission between humans can occur through heredity (as is often the case with GSS and CJD) or by contact with contaminated tissue, as might occur during a blood transfusion or organ transplant. There is no evidence for transmission via casual contact with an infected person. [\[link\]](#) lists TSEs that affect humans and their modes of transmission.

Transmissible Spongiform Encephalopathies (TSEs) in Humans	
Disease	Mechanism(s) of Transmission[footnote] National Institute of Neurological Disorders and Stroke. “Creutzfeldt-Jakob Disease Fact Sheet.” http://www.ninds.nih.gov/disorders/cjd/detail_cjd.htm (accessed December 31, 2015).
Sporadic CJD (sCJD)	Not known; possibly by alteration of normal prior protein (PrP) to rogue form due to somatic mutation

Transmissible Spongiform Encephalopathies (TSEs) in Humans	
Disease	Mechanism(s) of Transmission^[footnote] National Institute of Neurological Disorders and Stroke. “Creutzfeldt-Jakob Disease Fact Sheet.” http://www.ninds.nih.gov/disorders/cjd/detail_cjd.htm (accessed December 31, 2015).
Variant CJD (vCJD)	Eating contaminated cattle products and by secondary bloodborne transmission
Familial CJD (fCJD)	Mutation in germline PrP gene
Iatrogenic CJD (iCJD)	Contaminated neurosurgical instruments, corneal graft, gonadotrophic hormone, and, secondarily, by blood transfusion
Kuru	Eating infected meat through ritualistic cannibalism
Gerstmann-Straussler-Scheinker disease (GSS)	Mutation in germline PrP gene
Fatal familial insomnia (FFI)	Mutation in germline PrP gene

Prions are extremely difficult to destroy because they are resistant to heat, chemicals, and radiation. Even standard sterilization procedures do not ensure the destruction of these particles. Currently, there is no treatment or cure for TSE disease, and contaminated meats or infected animals must be handled according to federal guidelines to prevent transmission.

Note:

- Does a prion have a genome?

Note:

For more information on the handling of animals and prion-contaminated materials, visit the guidelines published on the [CDC](#) and [WHO](#) websites.

Note:**Resolution**

A few days later, David's doctor receives the results of the immunofluorescence test on his skin sample. The test is negative for rabies antigen. A second viral antigen test on his saliva sample also comes back negative. Despite these results, the doctor decides to continue David's current course of treatment. Given the positive RT-PCR test, it is best not to rule out a possible rabies infection.

Near the site of the bite, David receives an injection of rabies immunoglobulin, which attaches to and inactivates any rabies virus that may be present in his tissues. Over the next 14 days, he receives a series of four rabies-specific vaccinations in the arm. These vaccines activate David's immune response and help his body recognize and fight the virus. Thankfully, with treatment, David symptoms improve and he makes a full recovery.

Not all rabies cases have such a fortunate outcome. In fact, rabies is usually fatal once the patient starts to exhibit symptoms, and postbite treatments are mainly palliative (i.e., sedation and pain management).

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Other acellular agents such as **viroids**, **virusoids**, and **prions** also cause diseases. Viroids consist of small, naked ssRNAs that cause diseases in plants. Virusoids are ssRNAs that require other helper viruses to establish

an infection. Prions are proteinaceous infectious particles that cause **transmissible spongiform encephalopathies**.

- Prions are extremely resistant to chemicals, heat, and radiation.
- There are no treatments for prion infection.

Multiple Choice

Exercise:

Problem: Which of these infectious agents do not have nucleic acid?

- A. viroids
- B. viruses
- C. bacteria
- D. prions

Solution:

D

Exercise:

Problem: Which of the following is true of prions?

- A. They can be inactivated by boiling at 100 °C.
- B. They contain a capsid.
- C. They are a rogue form of protein, PrP.
- D. They can be reliably inactivated by an autoclave.

Solution:

C

Fill in the Blank

Exercise:

Problem:

Both viroids and virusoids have a(n) _____ genome, but virusoids require a(n) _____ to reproduce.

Solution:

RNA, helper virus

Short Answer**Exercise:****Problem:**

Describe the disease symptoms observed in animals infected with prions.

Critical Thinking**Exercise:**

Problem: Does a prion replicate? Explain.

Introduction

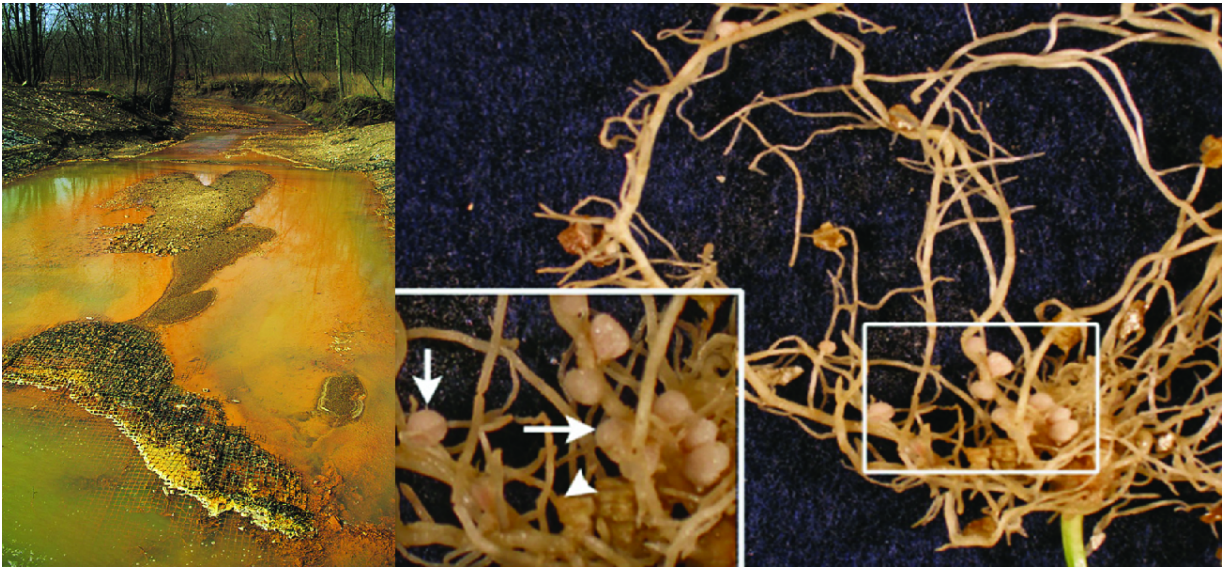
class="introduction"

Prokaryotes have great metabolic diversity with important consequences to other forms of life. Acidic mine drainage (left) is a serious environmental problem resulting from the introduction of water and oxygen to sulfide-oxidizing bacteria during mining processes.

These bacteria produce large amounts of sulfuric acid as a byproduct of their metabolism, resulting in a low-pH

environment
that can kill
many aquatic
plants and
animals. On
the other
hand, some
prokaryotes
are essential
to other life
forms. Root
nodules of
many plants
(right) house
nitrogen-
fixing
bacteria that
convert
atmospheric
nitrogen into
ammonia,
providing a
usable
nitrogen
source for
these plants.
(credit left:
modification
of work by D.
Hardesty,
USGS
Columbia
Environment
Research
Center; credit
right:
modification

of work by
Celmow SR,
Clairmont L,
Madsen LH,
and Guinel
FC)



Throughout earth's history, microbial metabolism has been a driving force behind the development and maintenance of the planet's biosphere. Eukaryotic organisms such as plants and animals typically depend on organic molecules for energy, growth, and reproduction. Prokaryotes, on the other hand, can metabolize a wide range of organic as well as inorganic matter, from complex organic molecules like cellulose to inorganic molecules and ions such as atmospheric nitrogen (N_2), molecular hydrogen (H_2), sulfide (S^{2-}), manganese (II) ions (Mn^{2+}), ferrous iron (Fe^{2+}), and ferric iron (Fe^{3+}), to name a few. By metabolizing such substances, microbes chemically convert them to other forms. In some cases, microbial metabolism produces chemicals that can be harmful to other organisms; in others, it produces substances that are essential to the metabolism and survival of other life forms ([\[link\]](#)).

LEARNING OBJECTIVES

- Define and describe metabolism
- Compare and contrast autotrophs and heterotrophs
- Describe the importance of oxidation-reduction reactions in metabolism
- Describe why ATP, FAD, NAD⁺, and NADP⁺ are important in a cell
- Identify the structure and structural components of an enzyme
- Describe the differences between competitive and noncompetitive enzyme inhibitors

Note:

Part 1

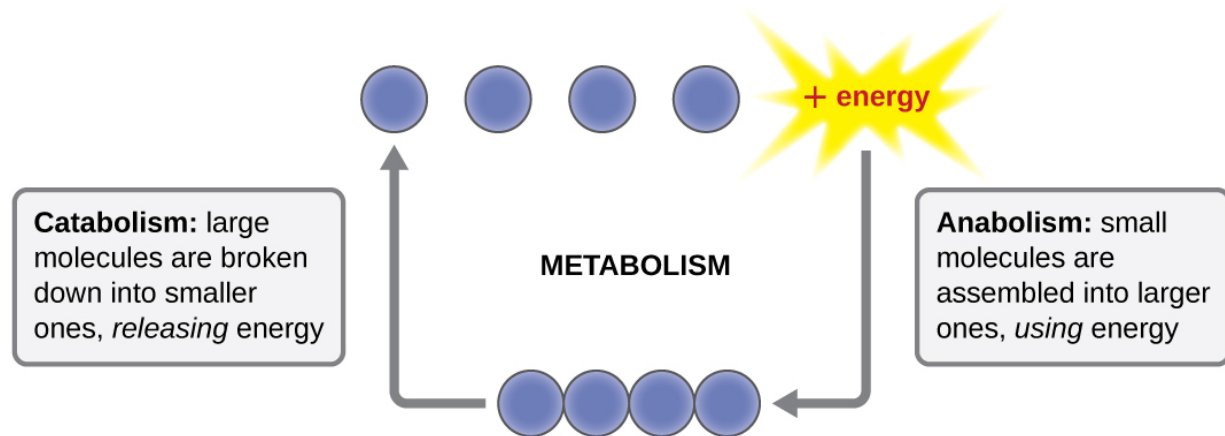
Hannah is a 15-month-old girl from Washington state. She is spending the summer in Gambia, where her parents are working for a nongovernmental organization. About 3 weeks after her arrival in Gambia, Hannah's appetite began to diminish and her parents noticed that she seemed unusually sluggish, fatigued, and confused. She also seemed very irritable when she was outdoors, especially during the day. When she began vomiting, her parents figured she had caught a 24-hour virus, but when her symptoms persisted, they took her to a clinic. The local physician noticed that Hannah's reflexes seemed abnormally slow, and when he examined her eyes with a light, she seemed unusually light sensitive. She also seemed to be experiencing a stiff neck.

- What are some possible causes of Hannah's symptoms?

Jump to the [next](#) Clinical Focus box.

The term used to describe all of the chemical reactions inside a cell is **metabolism** ([link](#)). Cellular processes such as the building or breaking down of complex molecules occur through series of stepwise, interconnected chemical reactions called metabolic pathways. Reactions that are spontaneous and release energy are **exergonic reactions**, whereas **endergonic reactions** require energy to proceed. The term **anabolism** refers to those endergonic metabolic pathways involved in biosynthesis, converting simple molecular building blocks into more complex molecules, and fueled by the use of cellular energy. Conversely, the term **catabolism** refers to exergonic pathways that break down complex molecules into simpler ones. Molecular energy stored in the bonds of complex molecules is released in catabolic pathways and harvested in

such a way that it can be used to produce high-energy molecules, which are used to drive anabolic pathways. Thus, in terms of energy and molecules, cells are continually balancing catabolism with anabolism.



Metabolism includes catabolism and anabolism. Anabolic pathways require energy to synthesize larger molecules. Catabolic pathways generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance.

Classification by Carbon and Energy Source

Organisms can be identified according to the source of carbon they use for metabolism as well as their energy source. The prefixes auto- ("self") and hetero- ("other") refer to the origins of the carbon sources various organisms can use. Organisms that convert inorganic carbon dioxide (CO_2) into organic carbon compounds are **autotrophs**. Plants and cyanobacteria are well-known examples of autotrophs. Conversely, **heterotrophs** rely on more complex organic carbon compounds as nutrients; these are provided to them initially by autotrophs. Many organisms, ranging from humans to many prokaryotes, including the well-studied *Escherichia coli*, are heterotrophic.

Organisms can also be identified by the energy source they use. All energy is derived from the transfer of electrons, but the source of electrons differs between various types of organisms. The prefixes photo- ("light") and chemo- ("chemical") refer to the energy sources that various organisms use. Those that get their energy for electron transfer from light are **phototrophs**, whereas **chemotrophs** obtain energy for electron transfer by breaking chemical bonds. There are two types of chemotrophs: **organotrophs** and **lithotrophs**. Organotrophs, including humans, fungi, and many prokaryotes, are chemotrophs that obtain energy from organic compounds. Lithotrophs ("litho" means "rock") are chemotrophs that get energy from inorganic compounds, including hydrogen sulfide (H_2S) and reduced iron. Lithotrophy is unique to the microbial world.

The strategies used to obtain both carbon and energy can be combined for the classification of organisms according to nutritional type. Most organisms are chemoheterotrophs because they

use organic molecules as both their electron and carbon sources. [\[link\]](#) summarizes this and the other classifications.

Classifications of Organisms by Energy and Carbon Source				
Classifications		Energy Source	Carbon Source	Examples
Chemotrophs	Chemoautotrophs	Chemical	Inorganic	Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide-oxidizing bacteria
	Chemoheterotrophs	Chemical	Organic compounds	All animals, most fungi, protozoa, and bacteria
Phototrophs	Photoautotrophs	Light	Inorganic	All plants, algae, cyanobacteria, and green and purple sulfur bacteria
	Photoheterotrophs	Light	Organic compounds	Green and purple nonsulfur bacteria, heliobacteria

Note:

- Explain the difference between catabolism and anabolism.
- Explain the difference between autotrophs and heterotrophs.

Oxidation and Reduction in Metabolism

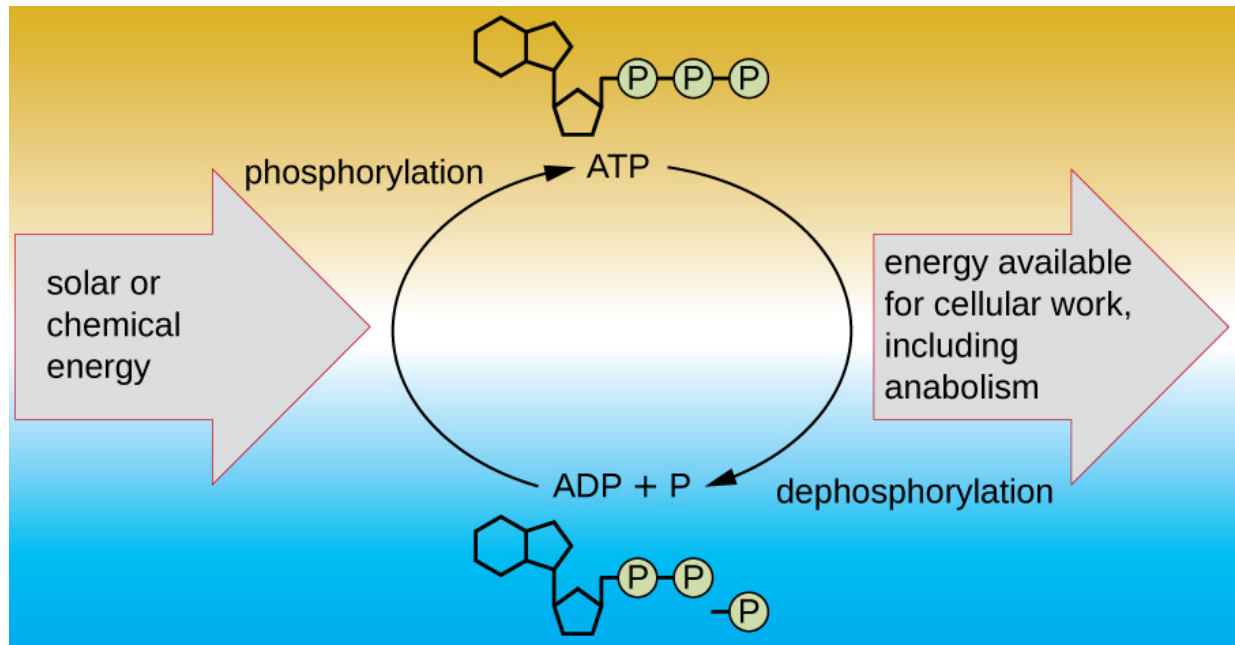
The transfer of electrons between molecules is important because most of the energy stored in atoms and used to fuel cell functions is in the form of high-energy electrons. The transfer of energy in the form of electrons allows the cell to transfer and use energy incrementally; that is, in small packages rather than a single, destructive burst. Reactions that remove electrons from donor molecules, leaving them oxidized, are **oxidation reactions**; those that add electrons to acceptor molecules, leaving them reduced, are **reduction reactions**. Because electrons can move from one molecule to another, oxidation and reduction occur in tandem. These pairs of reactions are called oxidation-reduction reactions, or **redox reactions**.

Energy Carriers: NAD^+ , NADP^+ , FAD, and ATP

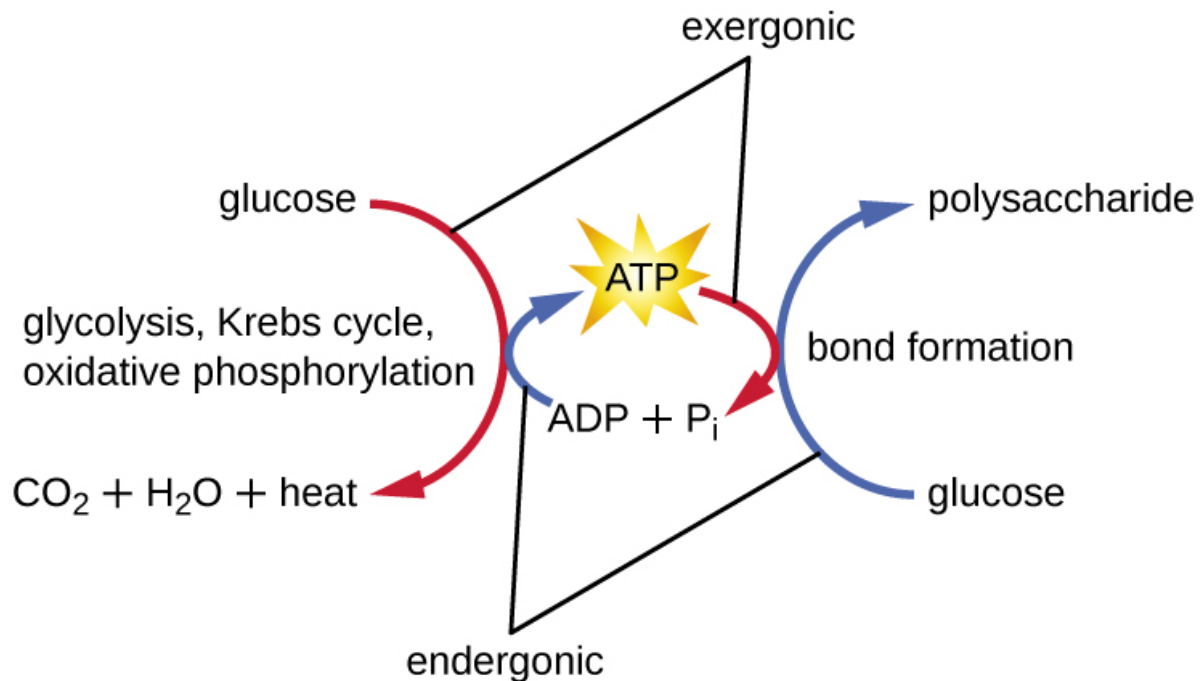
The energy released from the breakdown of the chemical bonds within nutrients can be stored either through the reduction of electron carriers or in the bonds of adenosine triphosphate (ATP). In living systems, a small class of compounds functions as mobile **electron carriers**, molecules that bind to and shuttle high-energy electrons between compounds in pathways. The principal electron carriers we will consider originate from the B vitamin group and are derivatives of nucleotides; they are **nicotinamide adenine dinucleotide**, **nicotine adenine dinucleotide phosphate**, and **flavin adenine dinucleotide**. These compounds can be easily reduced or oxidized. Nicotinamide adenine dinucleotide (NAD^+/NADH) is the most common mobile electron carrier used in catabolism. NAD^+ is the oxidized form of the molecule; NADH is the reduced form of the molecule. Nicotine adenine dinucleotide phosphate (NADP^+), the oxidized form of an NAD^+ variant that contains an extra phosphate group, is another important electron carrier; it forms **NADPH** when reduced. The oxidized form of flavin adenine dinucleotide is **FAD**, and its reduced form is **FADH₂**. Both NAD^+/NADH and FAD/FADH_2 are extensively used in energy extraction from sugars during catabolism in chemoheterotrophs, whereas $\text{NADP}^+/\text{NADPH}$ plays an important role in anabolic reactions and photosynthesis. Collectively, FADH_2 , NADH, and NADPH are often referred to as having reducing power due to their ability to donate electrons to various chemical reactions.

A living cell must be able to handle the energy released during catabolism in a way that enables the cell to store energy safely and release it for use only as needed. Living cells accomplish this by using the compound **adenosine triphosphate (ATP)**. ATP is often called the “energy currency” of the cell, and, like currency, this versatile compound can be used to fill any energy need of the cell. At the heart of ATP is a molecule of **adenosine monophosphate (AMP)**, which is composed of an adenine molecule bonded to a ribose molecule and a single phosphate group. Ribose is a five-carbon sugar found in RNA, and AMP is one of the nucleotides in RNA. The addition of a second phosphate group to this core molecule results in the formation of **adenosine diphosphate (ADP)**; the addition of a third phosphate group forms ATP ([link](#)). Adding a phosphate group to a molecule, a process called phosphorylation, requires energy. Phosphate groups are negatively charged and thus repel one another when they are arranged in series, as they are in ADP and ATP. This repulsion makes the ADP and ATP molecules inherently unstable. Thus, the bonds between phosphate groups (one in ADP and two in ATP) are called **high-energy phosphate bonds**. When these high-energy bonds are broken to release one phosphate (called **inorganic phosphate [P_i]**) or two connected

phosphate groups (called **pyrophosphate [PP_i]**) from ATP through a process called dephosphorylation, energy is released to drive endergonic reactions ([link](#)).



The energy released from dephosphorylation of ATP is used to drive cellular work, including anabolic pathways. ATP is regenerated through phosphorylation, harnessing the energy found in chemicals or from sunlight. (credit: modification of work by Robert Bear, David Rintoul)



Exergonic reactions are coupled to endergonic ones, making the combination favorable. Here, the endergonic reaction of ATP phosphorylation is coupled to the exergonic reactions of catabolism. Similarly, the exergonic reaction of ATP dephosphorylation is coupled to the endergonic reaction of polysaccharide formation, an example of anabolism.

Note:

- What is the function of an electron carrier?

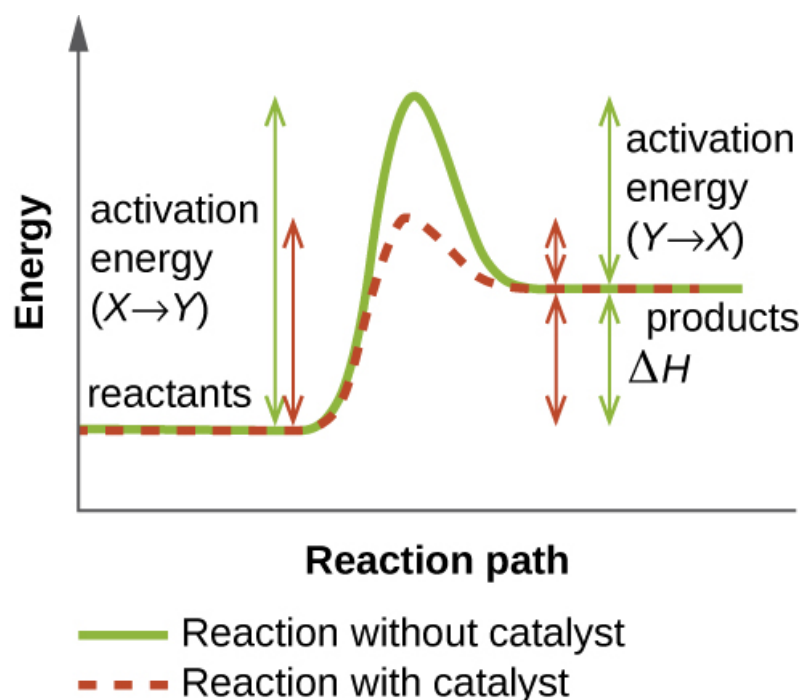
Enzyme Structure and Function

A substance that helps speed up a chemical reaction is a **catalyst**. Catalysts are not used or changed during chemical reactions and, therefore, are reusable. Whereas inorganic molecules may serve as catalysts for a wide range of chemical reactions, proteins called **enzymes** serve as catalysts for biochemical reactions inside cells. Enzymes thus play an important role in controlling cellular metabolism.

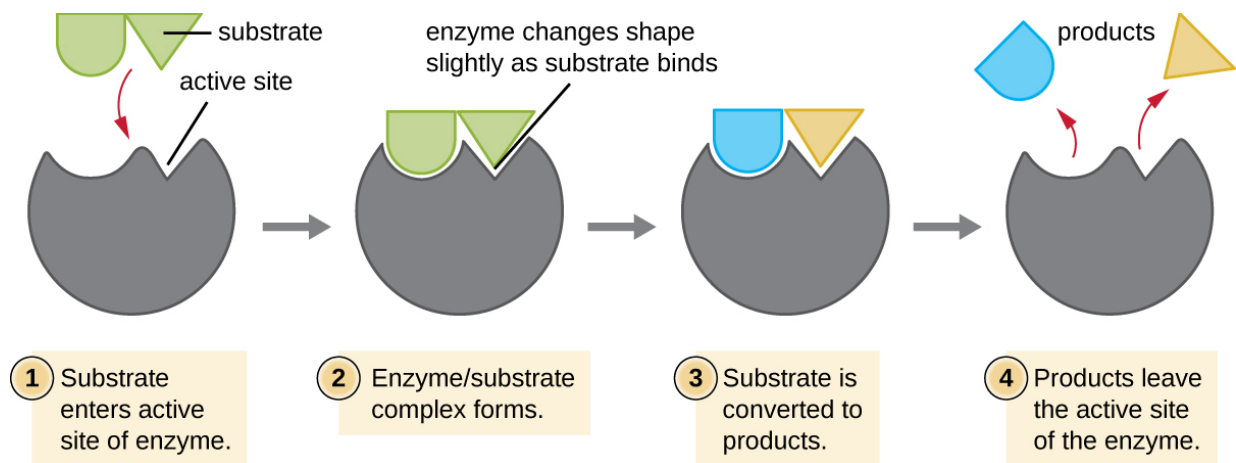
An enzyme functions by lowering the **activation energy** of a chemical reaction inside the cell. Activation energy is the energy needed to form or break chemical bonds and convert reactants

to products ([link](#)). Enzymes lower the activation energy by binding to the reactant molecules and holding them in such a way as to speed up the reaction.

The chemical reactants to which an enzyme binds are called **substrates**, and the location within the enzyme where the substrate binds is called the enzyme's **active site**. The characteristics of the amino acids near the active site create a very specific chemical environment within the active site that induces suitability to binding, albeit briefly, to a specific substrate (or substrates). Due to this jigsaw puzzle-like match between an enzyme and its substrates, enzymes are known for their specificity. In fact, as an enzyme binds to its substrate(s), the enzyme structure changes slightly to find the best fit between the transition state (a structural intermediate between the substrate and product) and the active site, just as a rubber glove molds to a hand inserted into it. This active-site modification in the presence of substrate, along with the simultaneous formation of the transition state, is called induced fit ([link](#)). Overall, there is a specifically matched enzyme for each substrate and, thus, for each chemical reaction; however, there is some flexibility as well. Some enzymes have the ability to act on several different structurally related substrates.



Enzymes lower the activation energy of a chemical reaction.



According to the induced-fit model, the active site of the enzyme undergoes conformational changes upon binding with the substrate.

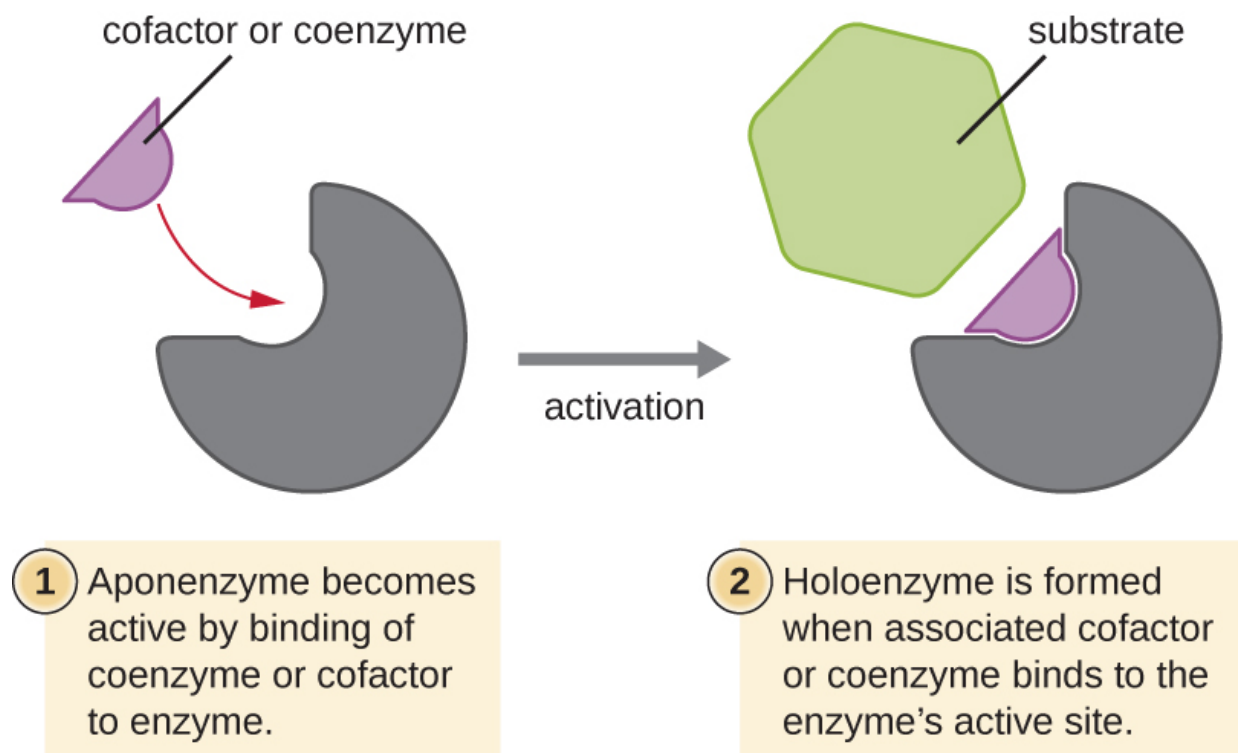
Enzymes are subject to influences by local environmental conditions such as pH, substrate concentration, and temperature. Although increasing the environmental temperature generally increases reaction rates, enzyme catalyzed or otherwise, increasing or decreasing the temperature outside of an optimal range can affect chemical bonds within the active site, making them less well suited to bind substrates. High temperatures will eventually cause enzymes, like other biological molecules, to denature, losing their three-dimensional structure and function. Enzymes are also suited to function best within a certain pH range, and, as with temperature, extreme environmental pH values (acidic or basic) can cause enzymes to denature. Active-site amino-acid side chains have their own acidic or basic properties that are optimal for catalysis and, therefore, are sensitive to changes in pH.

Another factor that influences enzyme activity is substrate concentration: Enzyme activity is increased at higher concentrations of substrate until it reaches a saturation point at which the enzyme can bind no additional substrate. Overall, enzymes are optimized to work best under the environmental conditions in which the organisms that produce them live. For example, while microbes that inhabit hot springs have enzymes that work best at high temperatures, human pathogens have enzymes that work best at 37°C. Similarly, while enzymes produced by most organisms work best at a neutral pH, microbes growing in acidic environments make enzymes optimized to low pH conditions, allowing for their growth at those conditions.

Many enzymes do not work optimally, or even at all, unless bound to other specific nonprotein helper molecules, either temporarily through ionic or hydrogen bonds or permanently through stronger covalent bonds. Binding to these molecules promotes optimal conformation and function for their respective enzymes. Two types of helper molecules are **cofactors** and **coenzymes**. Cofactors are inorganic ions such as iron (Fe^{2+}) and magnesium (Mg^{2+}) that help stabilize enzyme conformation and function. One example of an enzyme that requires a metal ion as a cofactor is the enzyme that builds DNA molecules, DNA polymerase, which requires a bound zinc ion (Zn^{2+}) to function.

Coenzymes are organic helper molecules that are required for enzyme action. Like enzymes, they are not consumed and, hence, are reusable. The most common sources of coenzymes are dietary vitamins. Some vitamins are precursors to coenzymes and others act directly as coenzymes.

Some cofactors and coenzymes, like coenzyme A (CoA), often bind to the enzyme's active site, aiding in the chemistry of the transition of a substrate to a product ([link](#)). In such cases, an enzyme lacking a necessary cofactor or coenzyme is called an **apoenzyme** and is inactive. Conversely, an enzyme with the necessary associated cofactor or coenzyme is called a **holoenzyme** and is active. NADH and ATP are also both examples of commonly used coenzymes that provide high-energy electrons or phosphate groups, respectively, which bind to enzymes, thereby activating them.



The binding of a coenzyme or cofactor to an apoenzyme is often required to form an active holoenzyme.

Note:

- What role do enzymes play in a chemical reaction?

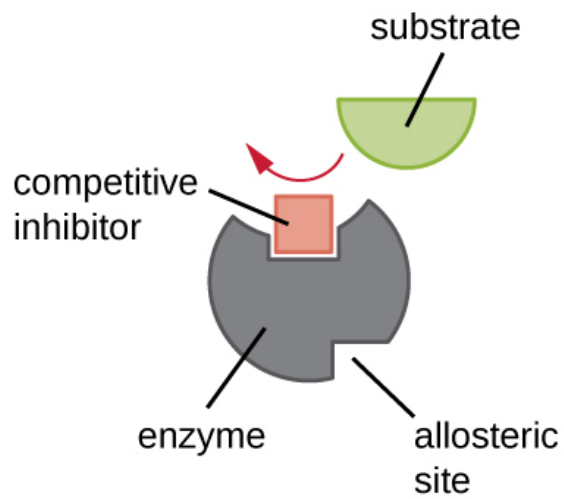
Enzyme Inhibitors

Enzymes can be regulated in ways that either promote or reduce their activity. There are many different kinds of molecules that inhibit or promote enzyme function, and various mechanisms exist for doing so ([\[link\]](#)). A **competitive inhibitor** is a molecule similar enough to a substrate that it can compete with the substrate for binding to the active site by simply blocking the substrate from binding. For a competitive inhibitor to be effective, the inhibitor concentration needs to be approximately equal to the substrate concentration. Sulfa drugs provide a good example of competitive competition. They are used to treat bacterial infections because they bind to the active site of an enzyme within the bacterial folic acid synthesis pathway. When present in a sufficient dose, a sulfa drug prevents folic acid synthesis, and bacteria are unable to grow because they cannot synthesize DNA, RNA, and proteins. Humans are unaffected because we obtain folic acid from our diets.

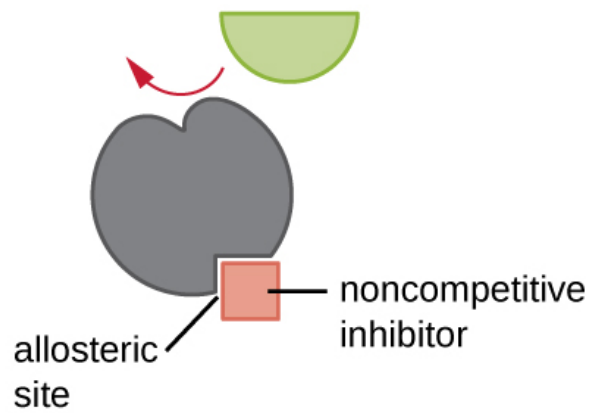
On the other hand, a **noncompetitive (allosteric) inhibitor** binds to the enzyme at an **allosteric site**, a location other than the active site, and still manages to block substrate binding to the active site by inducing a conformational change that reduces the affinity of the enzyme for its substrate ([\[link\]](#)). Because only one inhibitor molecule is needed per enzyme for effective inhibition, the concentration of inhibitors needed for noncompetitive inhibition is typically much lower than the substrate concentration.

In addition to allosteric inhibitors, there are **allosteric activators** that bind to locations on an enzyme away from the active site, inducing a conformational change that increases the affinity of the enzyme's active site(s) for its substrate(s).

Allosteric control is an important mechanism of regulation of metabolic pathways involved in both catabolism and anabolism. In a most efficient and elegant way, cells have evolved also to use the products of their own metabolic reactions for **feedback inhibition** of enzyme activity. Feedback inhibition involves the use of a pathway product to regulate its own further production. The cell responds to the abundance of specific products by slowing production during anabolic or catabolic reactions ([\[link\]](#)).

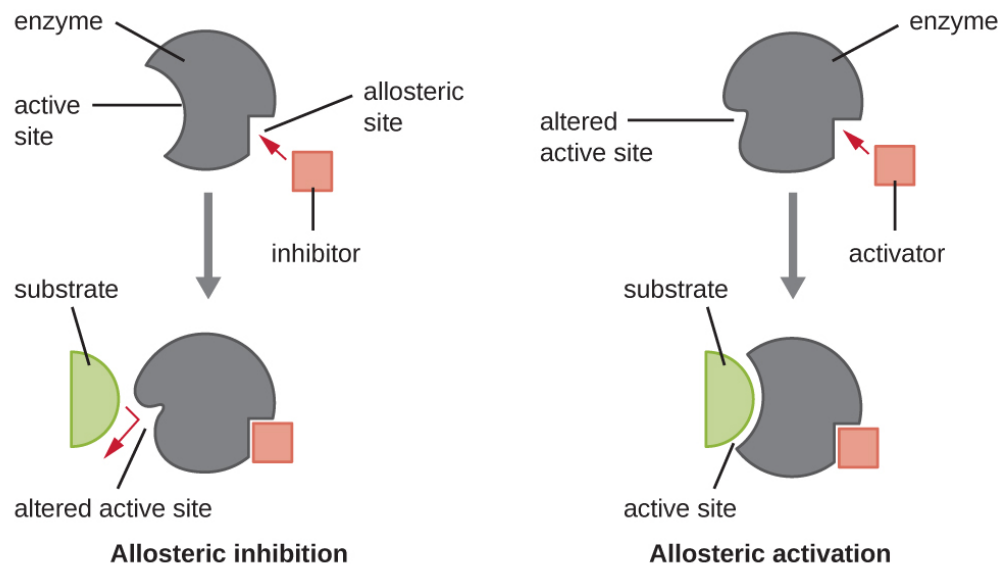


Competitive inhibition

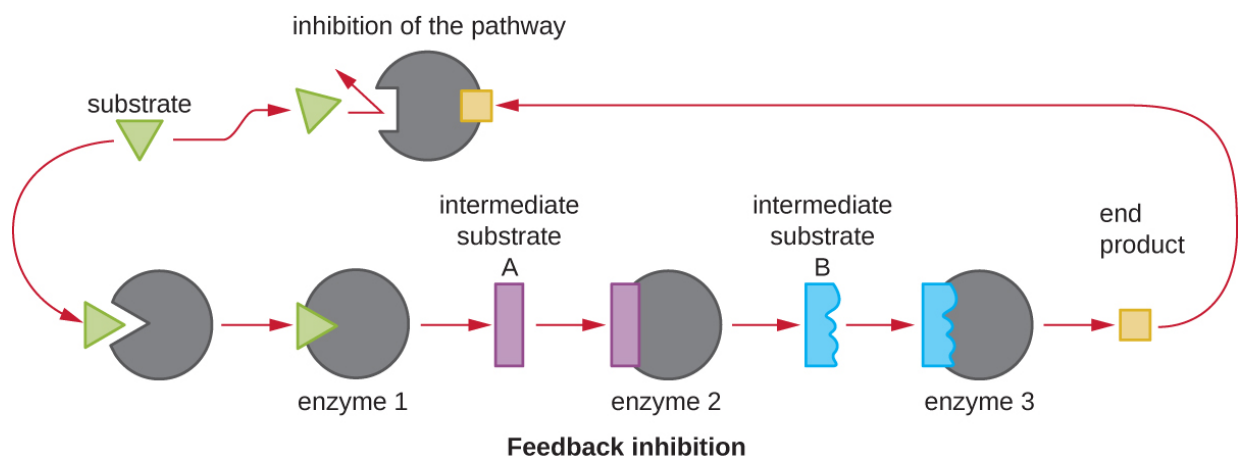


Noncompetitive inhibition

Enzyme activity can be regulated by either competitive inhibitors, which bind to the active site, or noncompetitive inhibitors, which bind to an allosteric site.



(a)



(b)

(a) Binding of an allosteric inhibitor reduces enzyme activity, but binding of an allosteric activator increases enzyme activity. (b) Feedback inhibition, where the end product of the pathway serves as a noncompetitive inhibitor to an enzyme early in the pathway, is an important mechanism of allosteric regulation in cells.

Note:

- Explain the difference between a competitive inhibitor and a noncompetitive inhibitor.

Key Concepts and Summary

- **Metabolism** includes chemical reactions that break down complex molecules (**catabolism**) and those that build complex molecules (**anabolism**).
- Organisms may be classified according to their source of carbon. **Autotrophs** convert inorganic carbon dioxide into organic carbon; **heterotrophs** use fixed organic carbon compounds.
- Organisms may also be classified according to their energy source. **Phototrophs** obtain their energy from light. **Chemotrophs** get their energy from chemical compounds. **Organotrophs** use organic molecules, and **lithotrophs** use inorganic chemicals.
- Cellular **electron carriers** accept high-energy electrons from foods and later serve as electron donors in subsequent **redox reactions**. **FAD/FADH₂**, **NAD⁺/NADH**, and **NADP⁺/NADPH** are important electron carriers.
- **Adenosine triphosphate (ATP)** serves as the energy currency of the cell, safely storing chemical energy in its two **high-energy phosphate bonds** for later use to drive processes requiring energy.
- **Enzymes** are biological **catalysts** that increase the rate of chemical reactions inside cells by lowering the activation energy required for the reaction to proceed.
- In nature, **exergonic reactions** do not require energy beyond activation energy to proceed, and they release energy. They may proceed without enzymes, but at a slow rate. Conversely, **endergonic reactions** require energy beyond activation energy to occur. In cells, endergonic reactions are coupled to exergonic reactions, making the combination energetically favorable.
- **Substrates** bind to the enzyme's **active site**. This process typically alters the structures of both the active site and the substrate, favoring transition-state formation; this is known as **induced fit**.
- **Cofactors** are inorganic ions that stabilize enzyme conformation and function. **Coenzymes** are organic molecules required for proper enzyme function and are often derived from vitamins. An enzyme lacking a cofactor or coenzyme is an **apoenzyme**; an enzyme with a bound cofactor or coenzyme is a **holoenzyme**.
- **Competitive inhibitors** regulate enzymes by binding to an enzyme's active site, preventing substrate binding. **Noncompetitive (allosteric) inhibitors** bind to **allosteric sites**, inducing a conformational change in the enzyme that prevents it from functioning. **Feedback inhibition** occurs when the product of a metabolic pathway noncompetitively binds to an enzyme early on in the pathway, ultimately preventing the synthesis of the product.

Multiple Choice

Exercise:

Problem:

Which of the following is an organism that obtains its energy from the transfer of electrons originating from chemical compounds and its carbon from an inorganic source?

A. chemoautotroph

- B. chemoheterotroph
- C. photoheterotroph
- D. photoautotroph

Solution:

A

Exercise:

Problem: Which of the following molecules is reduced?

- A. NAD^+
- B. FAD
- C. O_2
- D. NADPH

Solution:

D

Exercise:

Problem: Enzymes work by which of the following?

- A. increasing the activation energy
- B. reducing the activation energy
- C. making exergonic reactions endergonic
- D. making endergonic reactions exergonic

Solution:

B

Exercise:

Problem:

To which of the following does a competitive inhibitor most structurally resemble?

- A. the active site
- B. the allosteric site
- C. the substrate
- D. a coenzyme

Solution:

C

Exercise:

Problem:

Which of the following are organic molecules that help enzymes work correctly?

- A. cofactors
- B. coenzymes
- C. holoenzymes
- D. apoenzymes

Solution:

B

Fill in the Blank

Exercise:

Problem:

Processes in which cellular energy is used to make complex molecules from simpler ones are described as _____.

Solution:

anabolic

Exercise:

Problem: The loss of an electron from a molecule is called _____.

Solution:

oxidation

Exercise:

Problem: The part of an enzyme to which a substrate binds is called the _____.

Solution:

active site

True/False

Exercise:

Problem: Competitive inhibitors bind to allosteric sites.

Solution:

False

Short Answer

Exercise:

Problem:

In cells, can an oxidation reaction happen in the absence of a reduction reaction? Explain.

Exercise:

Problem:

What is the function of molecules like NAD^+/NADH and FAD/FADH_2 in cells?

Catabolism of Carbohydrates

LEARNING OBJECTIVES

- Describe why glycolysis is not oxygen dependent
- Define and describe the net yield of three-carbon molecules, ATP, and NADH from glycolysis
- Explain how three-carbon pyruvate molecules are converted into two-carbon acetyl groups that can be funneled into the Krebs cycle.
- Define and describe the net yield of CO₂, GTP/ATP, FADH₂, and NADH from the Krebs cycle
- Explain how intermediate carbon molecules of the Krebs cycle can be used in a cell

Extensive enzyme pathways exist for breaking down carbohydrates to capture energy in ATP bonds. In addition, many catabolic pathways produce intermediate molecules that are also used as building blocks for anabolism. Understanding these processes is important for several reasons. First, because the main metabolic processes involved are common to a wide range of chemoheterotrophic organisms, we can learn a great deal about human metabolism by studying metabolism in more easily manipulated bacteria like *E. coli*. Second, because animal and human pathogens are also chemoheterotrophs, learning about the details of metabolism in these bacteria, including possible differences between bacterial and human pathways, is useful for the diagnosis of pathogens as well as for the discovery of antimicrobial therapies targeting specific pathogens. Last, learning specifically about the pathways involved in chemoheterotrophic metabolism also serves as a basis for comparing other more unusual

metabolic strategies used by microbes. Although the chemical source of electrons initiating electron transfer is different between chemoheterotrophs and chemoautotrophs, many similar processes are used in both types of organisms.

The typical example used to introduce concepts of metabolism to students is carbohydrate catabolism. For chemoheterotrophs, our examples of metabolism start with the catabolism of polysaccharides such as glycogen, starch, or cellulose. Enzymes such as amylase, which breaks down glycogen or starch, and cellulases, which break down cellulose, can cause the hydrolysis of glycosidic bonds between the glucose monomers in these polymers, releasing glucose for further catabolism.

Glycolysis

For bacteria, eukaryotes, and most archaea, **glycolysis** is the most common pathway for the catabolism of glucose; it produces energy, reduced electron carriers, and precursor molecules for cellular metabolism. Every living organism carries out some form of glycolysis, suggesting this mechanism is an ancient universal metabolic process. The process itself does not use oxygen; however, glycolysis can be coupled with additional metabolic processes that are either aerobic or anaerobic. Glycolysis takes place in the cytoplasm of prokaryotic and eukaryotic cells. It begins with a single six-carbon glucose molecule and ends with two molecules of a three-carbon sugar called pyruvate. Pyruvate may be broken down further after glycolysis to harness more energy through aerobic or anaerobic respiration, but many organisms, including many microbes, may be unable to respire; for these organisms, glycolysis may be their only source of generating ATP.

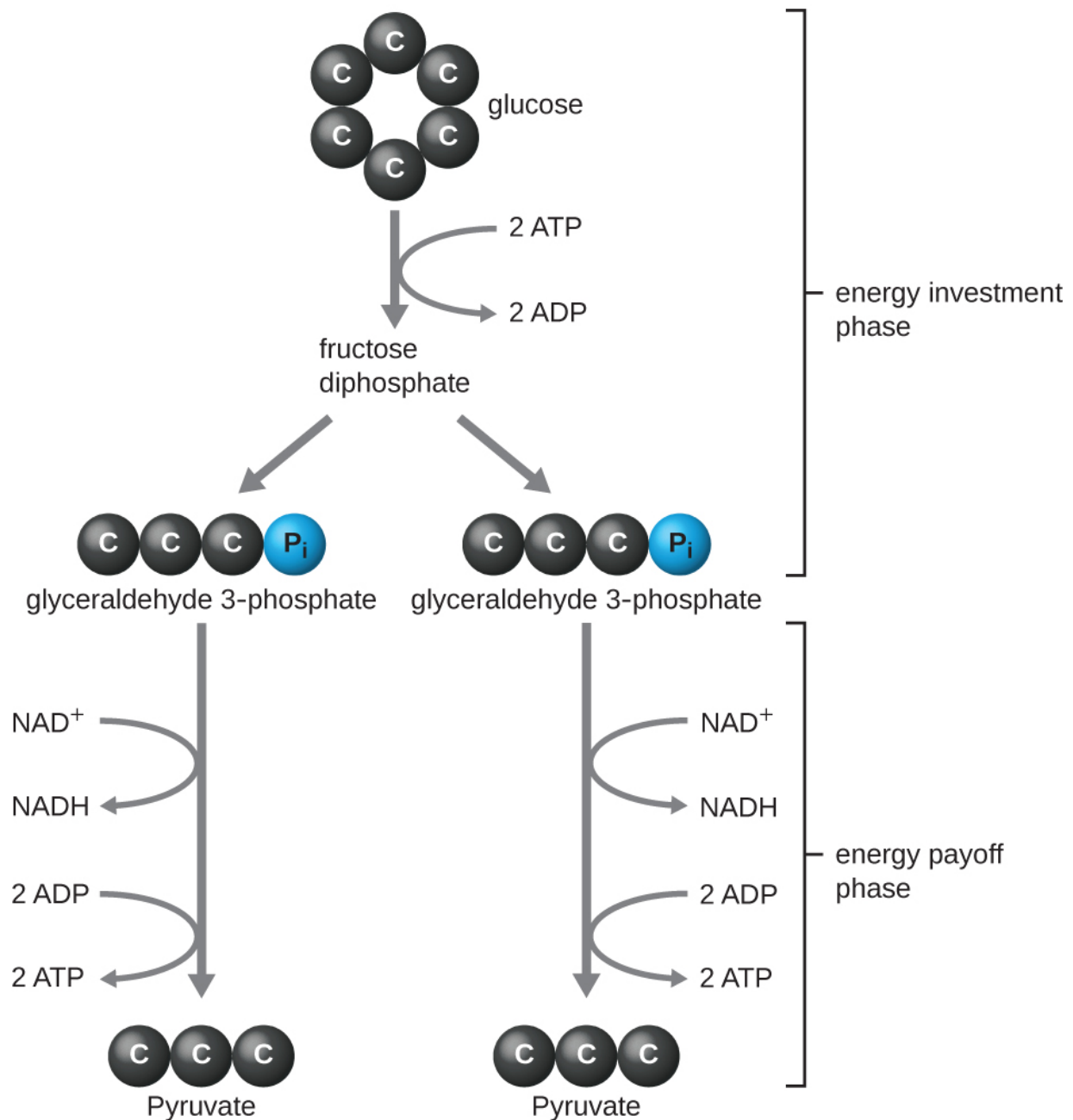
The type of glycolysis found in animals and that is most common in microbes is the **Embden-Meyerhof-Parnas (EMP) pathway**, named after Gustav Embden (1874–1933), Otto Meyerhof (1884–1951), and Jakub Parnas (1884–1949). Glycolysis using the EMP pathway consists of two distinct phases ([\[link\]](#)). The first part of the pathway, called the energy investment phase, uses energy from two ATP molecules to modify a glucose molecule so that the six-carbon sugar molecule can be split evenly into two phosphorylated three-carbon molecules called glyceraldehyde 3-phosphate

(G3P). The second part of the pathway, called the energy payoff phase, extracts energy by oxidizing G3P to pyruvate, producing four ATP molecules and reducing two molecules of NAD^+ to two molecules of NADH, using electrons that originated from glucose. (A discussion and illustration of the full EMP pathway with chemical structures and enzyme names appear in [Appendix C](#).)

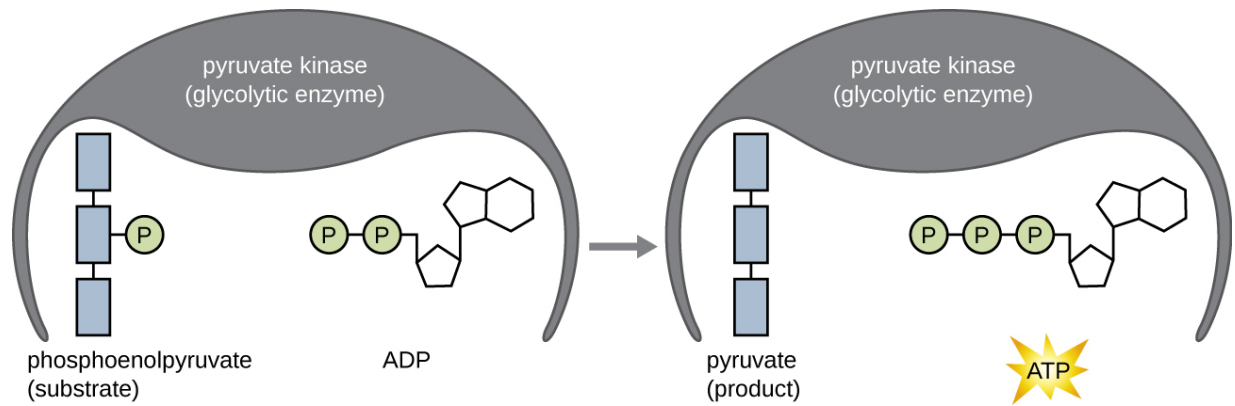
The ATP molecules produced during the energy payoff phase of glycolysis are formed by **substrate-level phosphorylation** ([link](#)), one of two mechanisms for producing ATP. In substrate-level phosphorylation, a phosphate group is removed from an organic molecule and is directly transferred to an available ADP molecule, producing ATP. During glycolysis, high-energy phosphate groups from the intermediate molecules are added to ADP to make ATP.

Overall, in this process of glycolysis, the net gain from the breakdown of a single glucose molecule is:

- two ATP molecules
- two NADH molecule, and
- two pyruvate molecules.



The energy investment phase of the Embden-Meyerhof-Parnas glycolysis pathway uses two ATP molecules to phosphorylate glucose, forming two glyceraldehyde 3-phosphate (G3P) molecules. The energy payoff phase harnesses the energy in the G3P molecules, producing four ATP molecules, two NADH molecules, and two pyruvates.



The ATP made during glycolysis is a result of substrate-level phosphorylation. One of the two enzymatic reactions in the energy payoff phase of Embden Meyerhof-Parnas glycolysis that produce ATP in this way is shown here.

Other Glycolytic Pathways

When we refer to glycolysis, unless otherwise indicated, we are referring to the EMP pathway used by animals and many bacteria. However, some prokaryotes use alternative glycolytic pathways. One important alternative is the **Entner-Doudoroff (ED) pathway**, named after its discoverers Nathan Entner and Michael Doudoroff (1911–1975). Although some bacteria, including the opportunistic gram-negative pathogen *Pseudomonas aeruginosa*, contain only the ED pathway for glycolysis, other bacteria, like *E. coli*, have the ability to use either the ED pathway or the EMP pathway.

A third type of glycolytic pathway that occurs in all cells, which is quite different from the previous two pathways, is the **pentose phosphate pathway** (PPP) also called the **phosphogluconate pathway** or the **hexose monophosphate shunt**. Evidence suggests that the PPP may be the most ancient universal glycolytic pathway. The intermediates from the PPP are used for the biosynthesis of nucleotides and amino acids. Therefore, this glycolytic pathway may be favored when the cell has need for nucleic acid and/or protein synthesis, respectively. A discussion and illustration of the

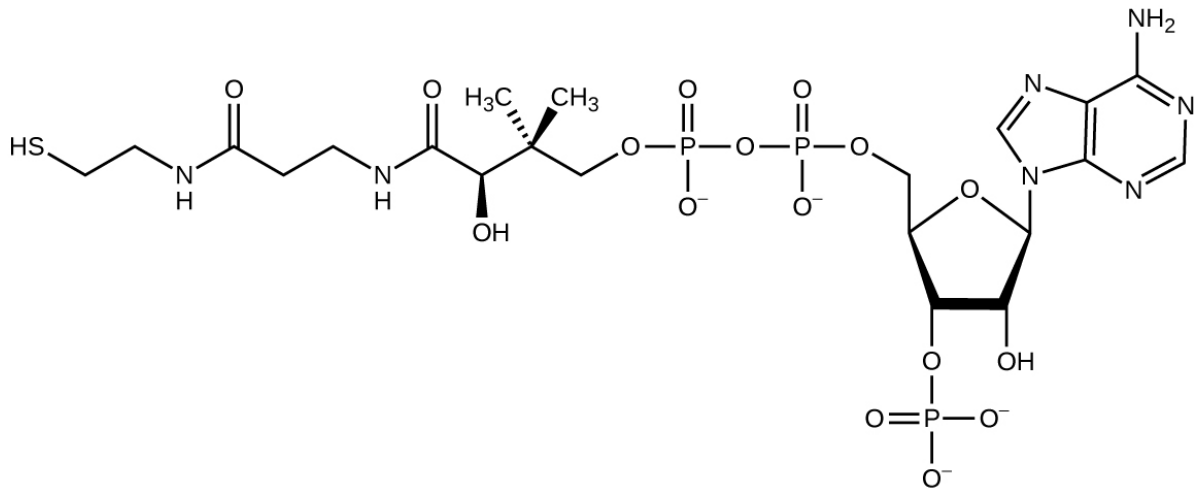
complete ED pathway and PPP with chemical structures and enzyme names appear in [Appendix C](#).

Note:

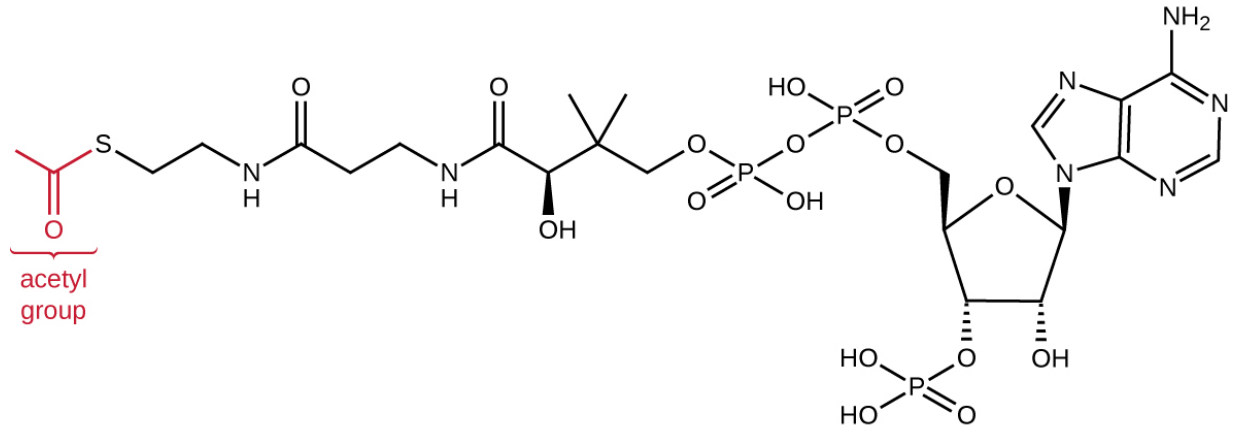
- When might an organism use the ED pathway or the PPP for glycolysis?

Transition Reaction, Coenzyme A, and the Krebs Cycle

Glycolysis produces pyruvate, which can be further oxidized to capture more energy. For pyruvate to enter the next oxidative pathway, it must first be decarboxylated by the enzyme complex pyruvate dehydrogenase to a two-carbon acetyl group in the **transition reaction**, also called the **bridge reaction** (see [Appendix C](#) and [\[link\]](#)). In the transition reaction, electrons are also transferred to NAD^+ to form NADH. To proceed to the next phase of this metabolic process, the comparatively tiny two-carbon acetyl must be attached to a very large carrier compound called coenzyme A (CoA). The transition reaction occurs in the mitochondrial matrix of eukaryotes; in prokaryotes, it occurs in the cytoplasm because prokaryotes lack membrane-enclosed organelles.



(a) coenzyme A without an attached acetyl group



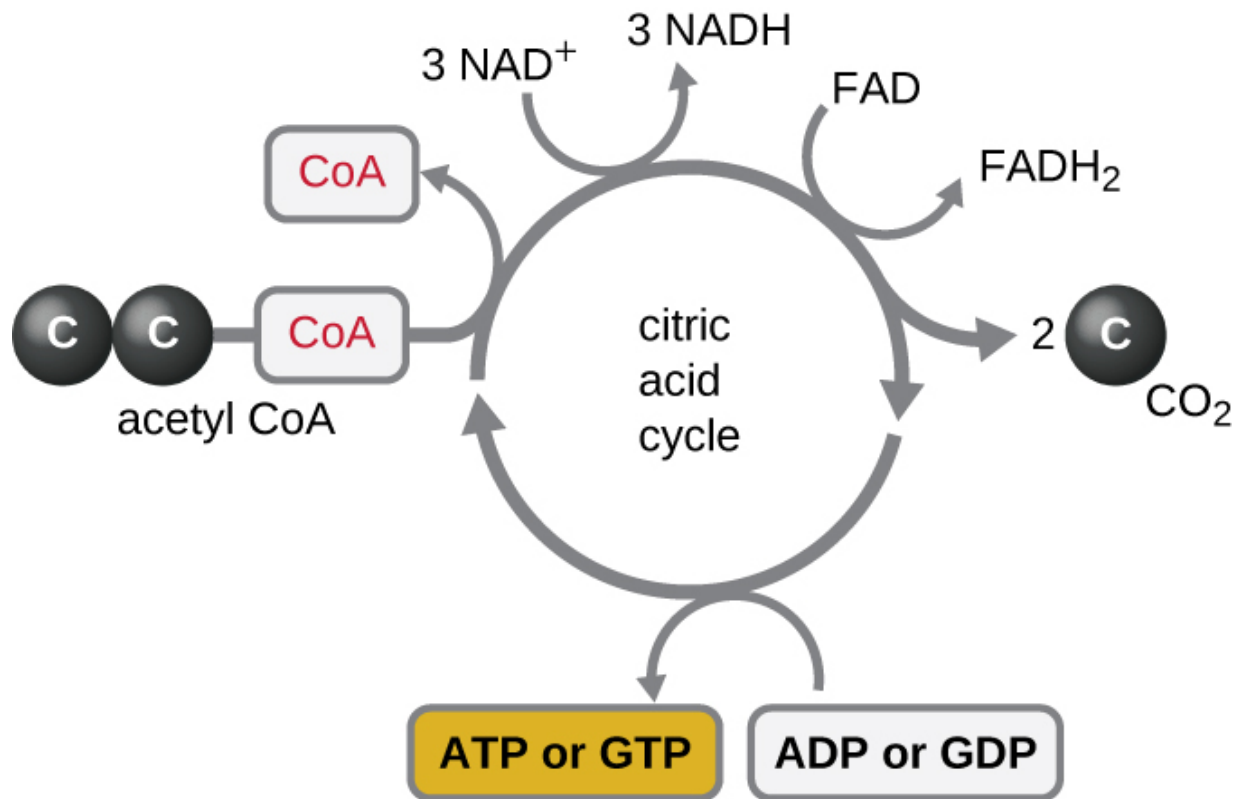
(b) coenzyme A with an attached acetyl group

(a) Coenzyme A is shown here without an attached acetyl group. (b) Coenzyme A is shown here with an attached acetyl group.

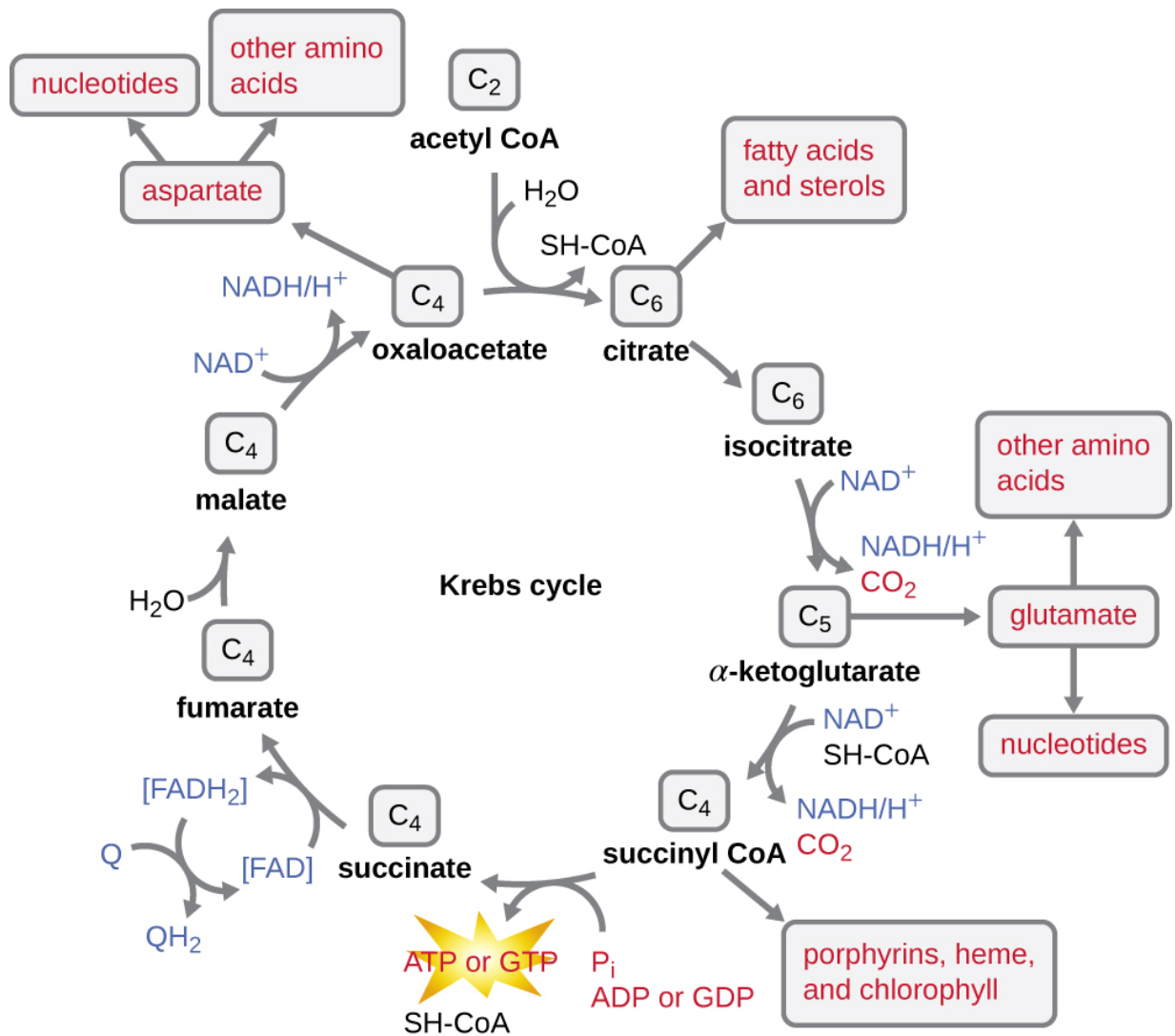
The **Krebs cycle** transfers remaining electrons from the acetyl group produced during the transition reaction to electron carrier molecules, thus reducing them. The Krebs cycle also occurs in the cytoplasm of prokaryotes along with glycolysis and the transition reaction, but it takes place in the mitochondrial matrix of eukaryotic cells where the transition reaction also occurs. The Krebs cycle is named after its discoverer, British scientist Hans Adolf Krebs (1900–1981) and is also called the **citric acid cycle**, or the **tricarboxylic acid cycle (TCA)** because citric acid has three carboxyl

groups in its structure. Unlike glycolysis, the Krebs cycle is a closed loop: The last part of the pathway regenerates the compound used in the first step ([\[link\]](#)). The eight steps of the cycle are a series of chemical reactions that capture the two-carbon acetyl group (the CoA carrier does not enter the Krebs cycle) from the transition reaction, which is added to a four-carbon intermediate in the Krebs cycle, producing the six-carbon intermediate citric acid (giving the alternate name for this cycle). As one turn of the cycle returns to the starting point of the four-carbon intermediate, the cycle produces two CO₂ molecules, one ATP molecule (or an equivalent, such as guanosine triphosphate [GTP]) produced by substrate-level phosphorylation, and three molecules of NADH and one of FADH₂. (A discussion and detailed illustration of the full Krebs cycle appear in [Appendix C.](#))

Although many organisms use the Krebs cycle as described as part of glucose metabolism, several of the intermediate compounds in the Krebs cycle can be used in synthesizing a wide variety of important cellular molecules, including amino acids, chlorophylls, fatty acids, and nucleotides; therefore, the cycle is both anabolic and catabolic ([\[link\]](#)).



The Krebs cycle, also known as the citric acid cycle, is summarized here. Note incoming two-carbon acetyl results in the main outputs per turn of two CO₂, three NADH, one FADH₂, and one ATP (or GTP) molecules made by substrate-level phosphorylation. Two turns of the Krebs cycle are required to process all of the carbon from one glucose molecule.



Many organisms use intermediates from the Krebs cycle, such as amino acids, fatty acids, and nucleotides, as building blocks for biosynthesis.

Key Concepts and Summary

- **Glycolysis** is the first step in the breakdown of glucose, resulting in the formation of ATP, which is produced by **substrate-level phosphorylation**; NADH; and two pyruvate molecules. Glycolysis does not use oxygen and is not oxygen dependent.

- After glycolysis, a three-carbon pyruvate is decarboxylated to form a two-carbon acetyl group, coupled with the formation of NADH. The acetyl group is attached to a large carrier compound called coenzyme A.
- After the transition step, coenzyme A transports the two-carbon acetyl to the **Krebs cycle**, where the two carbons enter the cycle. Per turn of the cycle, one acetyl group derived from glycolysis is further oxidized, producing three NADH molecules, one FADH₂, and one ATP by **substrate-level phosphorylation**, and releasing two CO₂ molecules.
- The Krebs cycle may be used for other purposes. Many of the intermediates are used to synthesize important cellular molecules, including amino acids, chlorophylls, fatty acids, and nucleotides.

Multiple Choice

Exercise:

Problem:

During which of the following is ATP not made by substrate-level phosphorylation?

- A. Embden-Meyerhof pathway
- B. Transition reaction
- C. Krebs cycle
- D. Entner-Doudoroff pathway

Solution:

B

Exercise:

Problem:

Which of the following products is made during Embden-Meyerhof glycolysis?

- A. NAD⁺
 - B. pyruvate
 - C. CO₂
 - D. two-carbon acetyl
-

Solution:

B

Exercise:

Problem:

During the catabolism of glucose, which of the following is produced only in the Krebs cycle?

- A. ATP
 - B. NADH
 - C. NADPH
 - D. FADH₂
-

Solution:

D

Exercise:

Problem:

Which of the following is not a name for the cycle resulting in the conversion of a two-carbon acetyl to one ATP, two CO₂, one FADH₂, and three NADH molecules?

- A. Krebs cycle
 - B. tricarboxylic acid cycle
 - C. Calvin cycle
 - D. citric acid cycle
-

Solution:

C

True/False

Exercise:

Problem:

Glycolysis requires oxygen or another inorganic final electron acceptor to proceed.

Solution:

False

Fill in the Blank

Exercise:

Problem:

Per turn of the Krebs cycle, one acetyl is oxidized, forming ____ CO₂, ____ ATP, ____ NADH, and ____ FADH₂ molecules.

Solution:

2; 1; 3; 1

Exercise:

Problem:

Most commonly, glycolysis occurs by the _____ pathway.

Solution:

Embden-Meyerhof

Short Answer

Exercise:

Problem:

What is substrate-level phosphorylation? When does it occur during the breakdown of glucose to CO_2 ?

Exercise:

Problem:

Why is the Krebs cycle important in both catabolism and anabolism?

Critical Thinking

Exercise:

Problem:

What would be the consequences to a cell of having a mutation that knocks out coenzyme A synthesis?

Cellular Respiration

LEARNING OBJECTIVES

- Compare and contrast the electron transport system location and function in a prokaryotic cell and a eukaryotic cell
- Compare and contrast the differences between substrate-level and oxidative phosphorylation
- Explain the relationship between chemiosmosis and proton motive force
- Describe the function and location of ATP synthase in a prokaryotic versus eukaryotic cell
- Compare and contrast aerobic and anaerobic respiration

We have just discussed two pathways in glucose catabolism—glycolysis and the Krebs cycle—that generate ATP by substrate-level phosphorylation. Most ATP, however, is generated during a separate process called **oxidative phosphorylation**, which occurs during cellular respiration. Cellular respiration begins when electrons are transferred from NADH and FADH₂—made in glycolysis, the transition reaction, and the Krebs cycle—through a series of chemical reactions to a final inorganic electron acceptor (either oxygen in aerobic respiration or non-oxygen inorganic molecules in anaerobic respiration). These electron transfers take place on the inner part of the cell membrane of prokaryotic cells or in specialized protein complexes in the inner membrane of the mitochondria of eukaryotic cells. The energy of the electrons is harvested to generate an electrochemical gradient across the membrane, which is used to make ATP by oxidative phosphorylation.

Electron Transport System

The **electron transport system (ETS)** is the last component involved in the process of cellular respiration; it comprises a series of membrane-associated protein complexes and associated mobile accessory electron carriers ([link](#)). Electron transport is a series of chemical reactions that resembles a bucket brigade in that electrons from NADH and FADH₂ are passed rapidly from one ETS electron carrier to the next. These carriers can pass electrons along in the ETS because of their **redox potential**. For a protein or chemical to accept electrons, it must have a more positive redox potential than the electron donor. Therefore, electrons move from electron carriers with more negative redox potential to those with more positive redox potential. The four major classes of electron carriers involved in both eukaryotic and prokaryotic electron transport systems are the cytochromes, flavoproteins, iron-sulfur proteins, and the quinones.

In **aerobic respiration**, the final electron acceptor (i.e., the one having the most positive redox potential) at the end of the ETS is an oxygen molecule (O₂) that becomes reduced to water (H₂O) by the final ETS carrier. This electron carrier, **cytochrome oxidase**, differs between bacterial types and can be used to differentiate closely related bacteria for diagnoses. For example, the gram-negative opportunist *Pseudomonas aeruginosa* and the gram-negative cholera-causing *Vibrio cholerae* use cytochrome c oxidase, which can be detected by the oxidase test, whereas other gram-negative Enterobacteriaceae, like *E. coli*, are negative for this test because they produce different cytochrome oxidase types.

There are many circumstances under which aerobic respiration is not possible, including any one or more of the following:

- The cell lacks genes encoding an appropriate cytochrome oxidase for transferring electrons to oxygen at the end of the electron transport system.
- The cell lacks genes encoding enzymes to minimize the severely damaging effects of dangerous oxygen radicals produced during aerobic respiration, such as hydrogen peroxide (H₂O₂) or superoxide (O₂⁻).

- The cell lacks a sufficient amount of oxygen to carry out aerobic respiration.

One possible alternative to aerobic respiration is **anaerobic respiration**, using an inorganic molecule other than oxygen as a final electron acceptor. There are many types of anaerobic respiration found in bacteria and archaea. Denitrifiers are important soil bacteria that use nitrate (NO_3^-) and nitrite (NO_2^-) as final electron acceptors, producing nitrogen gas (N_2). Many aerobically respiring bacteria, including *E. coli*, switch to using nitrate as a final electron acceptor and producing nitrite when oxygen levels have been depleted.

Microbes using anaerobic respiration commonly have an intact Krebs cycle, so these organisms can access the energy of the NADH and FADH_2 molecules formed. However, anaerobic respirers use altered ETS carriers encoded by their genomes, including distinct complexes for electron transfer to their final electron acceptors. Smaller electrochemical gradients are generated from these electron transfer systems, so less ATP is formed through anaerobic respiration.

Note:

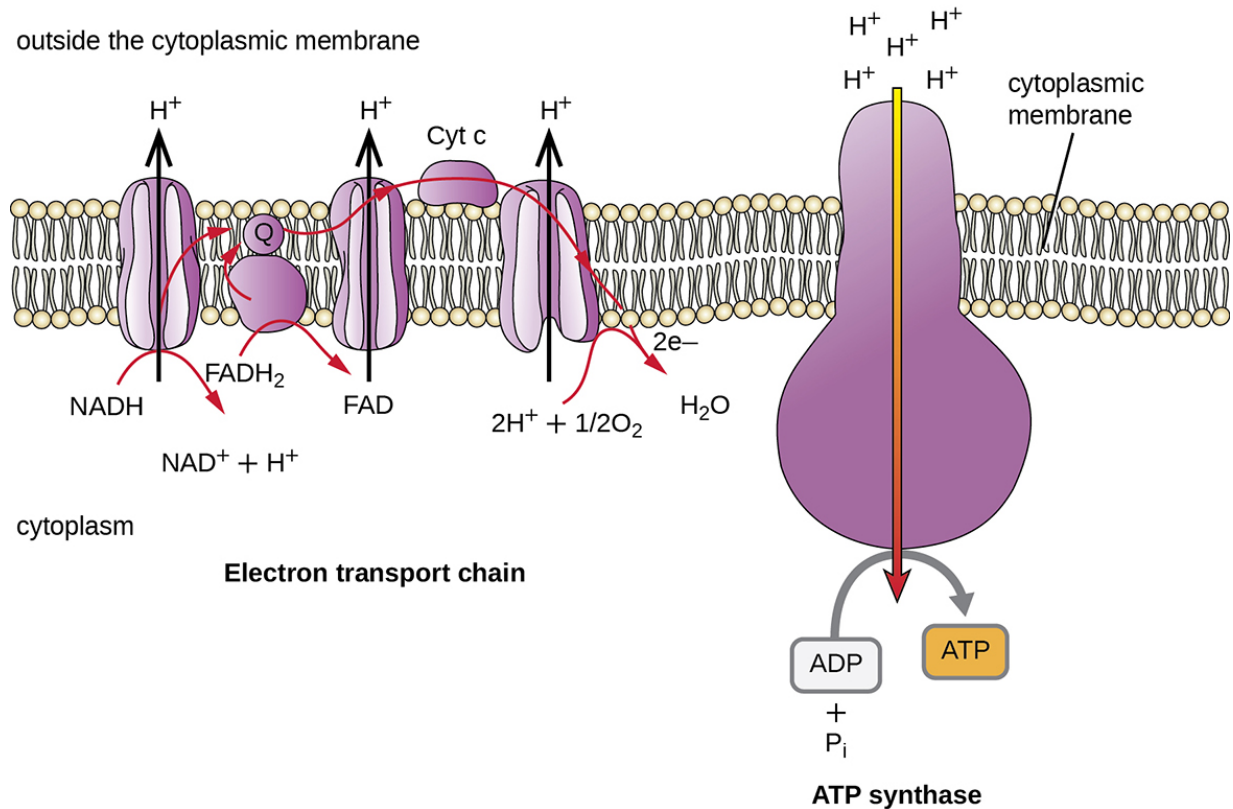
- Do both aerobic respiration and anaerobic respiration use an electron transport chain?

Chemiosmosis, Proton Motive Force, and Oxidative Phosphorylation

In each transfer of an electron through the ETS, the electron loses energy, but with some transfers, the energy is stored as potential energy by using it to pump hydrogen ions (H^+) across a membrane. In prokaryotic cells, H^+ is pumped to the outside of the cytoplasmic membrane (called the periplasmic space in gram-negative and gram-positive bacteria), and in eukaryotic cells,

they are pumped from the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space. There is an uneven distribution of H^+ across the membrane that establishes an electrochemical gradient because H^+ ions are positively charged (electrical) and there is a higher concentration (chemical) on one side of the membrane. This electrochemical gradient formed by the accumulation of H^+ (also known as a proton) on one side of the membrane compared with the other is referred to as the **proton motive force** (PMF). Because the ions involved are H^+ , a pH gradient is also established, with the side of the membrane having the higher concentration of H^+ being more acidic. Beyond the use of the PMF to make ATP, as discussed in this chapter, the PMF can also be used to drive other energetically unfavorable processes, including nutrient transport and flagella rotation for motility.

The potential energy of this electrochemical gradient generated by the ETS causes the H^+ to diffuse across a membrane (the plasma membrane in prokaryotic cells and the inner membrane in mitochondria in eukaryotic cells). This flow of hydrogen ions across the membrane, called **chemiosmosis**, must occur through a channel in the membrane via a membrane-bound enzyme complex called **ATP synthase** ([\[link\]](#)). The tendency for movement in this way is much like water accumulated on one side of a dam, moving through the dam when opened. ATP synthase (like a combination of the intake and generator of a hydroelectric dam) is a complex protein that acts as a tiny generator, turning by the force of the H^+ diffusing through the enzyme, down their electrochemical gradient from where there are many mutually repelling H^+ to where there are fewer H^+ . In prokaryotic cells, H^+ flows from the outside of the cytoplasmic membrane into the cytoplasm, whereas in eukaryotic mitochondria, H^+ flows from the intermembrane space to the mitochondrial matrix. The turning of the parts of this molecular machine regenerates ATP from ADP and inorganic phosphate (P_i) by oxidative phosphorylation, a second mechanism for making ATP that harvests the potential energy stored within an electrochemical gradient.



The bacterial electron transport chain is a series of protein complexes, electron carriers, and ion pumps that is used to pump H^+ out of the bacterial cytoplasm into the extracellular space. H^+ flows back down the electrochemical gradient into the bacterial cytoplasm through ATP synthase, providing the energy for ATP production by oxidative phosphorylation. (credit: modification of work by Klaus Hoffmeier)

The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport system complexes can pump through the membrane varies between different species of organisms. In aerobic respiration in mitochondria, the passage of electrons from one molecule of NADH generates enough proton motive force to make three ATP molecules by oxidative phosphorylation, whereas the passage of electrons from one molecule of $FADH_2$ generates enough proton motive force to make only two ATP molecules. Thus, the 10 NADH molecules made per glucose during glycolysis, the transition

reaction, and the Krebs cycle carry enough energy to make 30 ATP molecules, whereas the two FADH_2 molecules made per glucose during these processes provide enough energy to make four ATP molecules. Overall, the theoretical maximum yield of ATP made during the complete aerobic respiration of glucose is 38 molecules, with four being made by substrate-level phosphorylation and 34 being made by oxidative phosphorylation ([\[link\]](#)). In reality, the total ATP yield is usually less, ranging from one to 34 ATP molecules, depending on whether the cell is using aerobic respiration or anaerobic respiration; in eukaryotic cells, some energy is expended to transport intermediates from the cytoplasm into the mitochondria, affecting ATP yield.

[\[link\]](#) summarizes the theoretical maximum yields of ATP from various processes during the complete aerobic respiration of one glucose molecule.

Source	Carbon Flow	Molecules of Reduced Coenzymes Produced	Net ATP Molecules Made by Substrate-Level Phosphorylation	Net ATP Molecules Made by Oxidative Phosphorylation	Theoretical Maximum Yield of ATP Molecules
Glycolysis (EMP)	Glucose (6C) $\longrightarrow$ 2 pyruvates (3C)	2 NADH	2 ATP	6 ATP from 2 NADH	8
Transition reaction	2 pyruvates (3C) $\longrightarrow$ 2 acetyl (2C) + 2 CO_2	2 NADH		6 ATP from 2 NADH	6
Krebs cycle	2 acetyl (2C) $\longrightarrow$ 4 CO_2	6 NADH 2 FADH_2	2 ATP	18 ATP from 6 NADH 4 ATP from 2 FADH_2	24
Total:	glucose (6C) $\longrightarrow$ 6 CO_2	10 NADH 2 FADH_2	4 ATP	34 ATP	38 ATP

Note:

- What are the functions of the proton motive force?

Key Concepts and Summary

- Most ATP generated during the cellular respiration of glucose is made by **oxidative phosphorylation**.
- An **electron transport system (ETS)** is composed of a series of membrane-associated protein complexes and associated mobile accessory electron carriers. The ETS is embedded in the cytoplasmic membrane of prokaryotes and the inner mitochondrial membrane of eukaryotes.
- Each ETS complex has a different redox potential, and electrons move from electron carriers with more negative redox potential to those with more positive redox potential.
- To carry out **aerobic respiration**, a cell requires oxygen as the final electron acceptor. A cell also needs a complete Krebs cycle, an appropriate cytochrome oxidase, and oxygen detoxification enzymes to prevent the harmful effects of oxygen radicals produced during aerobic respiration.
- Organisms performing **anaerobic respiration** use alternative electron transport system carriers for the ultimate transfer of electrons to the final non-oxygen electron acceptors.
- Microbes show great variation in the composition of their electron transport systems, which can be used for diagnostic purposes to help identify certain pathogens.
- As electrons are passed from NADH and FADH₂ through an ETS, the electron loses energy. This energy is stored through the pumping of H⁺ across the membrane, generating a **proton motive force**.
- The energy of this proton motive force can be harnessed by allowing hydrogen ions to diffuse back through the membrane by **chemiosmosis** using **ATP synthase**. As hydrogen ions diffuse through down their electrochemical gradient, components of ATP synthase spin, making ATP from ADP and P_i by oxidative phosphorylation.

- Aerobic respiration forms more ATP (a maximum of 34 ATP molecules) during oxidative phosphorylation than does anaerobic respiration (between one and 32 ATP molecules).

Multiple Choice

Exercise:

Problem:

Which is the location of electron transports systems in prokaryotes?

- A. the outer mitochondrial membrane
- B. the cytoplasm
- C. the inner mitochondrial membrane
- D. the cytoplasmic membrane

Solution:

D

Exercise:

Problem:

Which is the source of the energy used to make ATP by oxidative phosphorylation?

- A. oxygen
- B. high-energy phosphate bonds
- C. the proton motive force
- D. P_i

Solution:

C

Exercise:

Problem:

A cell might perform anaerobic respiration for which of the following reasons?

- A. It lacks glucose for degradation.
- B. It lacks the transition reaction to convert pyruvate to acetyl-CoA.
- C. It lacks Krebs cycle enzymes for processing acetyl-CoA to CO₂.
- D. It lacks a cytochrome oxidase for passing electrons to oxygen.

Solution:

D

Exercise:

Problem: In prokaryotes, which of the following is true?

- A. As electrons are transferred through an ETS, H⁺ is pumped out of the cell.
- B. As electrons are transferred through an ETS, H⁺ is pumped into the cell.
- C. As protons are transferred through an ETS, electrons are pumped out of the cell.
- D. As protons are transferred through an ETS, electrons are pumped into the cell.

Solution:

A

Exercise:**Problem:**

Which of the following is not an electron carrier within an electron transport system?

- A. flavoprotein
 - B. ATP synthase
 - C. ubiquinone
 - D. cytochrome oxidase
-

Solution:

B

Fill in the Blank

Exercise:

Problem:

The final ETS complex used in aerobic respiration that transfers energy-depleted electrons to oxygen to form H₂O is called _____.

Solution:

cytochrome oxidase

Exercise:

Problem:

The passage of hydrogen ions through _____ down their electrochemical gradient harnesses the energy needed for ATP synthesis by oxidative phosphorylation.

Solution:

ATP synthase

True/False

Exercise:

Problem:

All organisms that use aerobic cellular respiration have cytochrome oxidase.

Solution:

True

Short Answer**Exercise:****Problem:**

What is the relationship between chemiosmosis and the proton motive force?

Exercise:**Problem:**

How does oxidative phosphorylation differ from substrate-level phosphorylation?

Exercise:**Problem:**

How does the location of ATP synthase differ between prokaryotes and eukaryotes? Where do protons accumulate as a result of the ETS in each cell type?

Fermentation

LEARNING OBJECTIVES

- Define fermentation and explain why it does not require oxygen
- Describe the fermentation pathways and their end products and give examples of microorganisms that use these pathways
- Compare and contrast fermentation and anaerobic respiration

Many cells are unable to carry out respiration because of one or more of the following circumstances:

1. The cell lacks a sufficient amount of any appropriate, inorganic, final electron acceptor to carry out cellular respiration.
2. The cell lacks genes to make appropriate complexes and electron carriers in the electron transport system.
3. The cell lacks genes to make one or more enzymes in the Krebs cycle.

Whereas lack of an appropriate inorganic final electron acceptor is environmentally dependent, the other two conditions are genetically determined. Thus, many prokaryotes, including members of the clinically important genus *Streptococcus*, are permanently incapable of respiration, even in the presence of oxygen. Conversely, many prokaryotes are facultative, meaning that, should the environmental conditions change to provide an appropriate inorganic final electron acceptor for respiration, organisms containing all the genes required to do so will switch to cellular respiration for glucose metabolism because respiration allows for much greater ATP production per glucose molecule.

If respiration does not occur, NADH must be reoxidized to NAD⁺ for reuse as an electron carrier for glycolysis, the cell's only mechanism for producing any ATP, to continue. Some living systems use an organic molecule (commonly pyruvate) as a final electron acceptor through a process called **fermentation**. Fermentation does not involve an electron transport system and does not directly produce any additional ATP beyond that produced during glycolysis by substrate-level phosphorylation. Organisms carrying out fermentation, called fermenters, produce a maximum of two ATP molecules per glucose during glycolysis. [\[link\]](#) compares the final electron acceptors and methods of ATP synthesis in aerobic respiration, anaerobic respiration, and fermentation. Note that the number of ATP molecules shown for glycolysis assumes the Embden-Meyerhof-Parnas pathway. The number of ATP molecules made by substrate-level phosphorylation (SLP) versus oxidative phosphorylation (OP) are indicated.

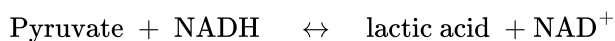
Comparison of Respiration Versus Fermentation				
Type of Metabolism	Example	Final Electron Acceptor	Pathways Involved in ATP Synthesis (Type of Phosphorylation)	Maximum Yield of ATP Molecules

Comparison of Respiration Versus Fermentation				
Type of Metabolism	Example	Final Electron Acceptor	Pathways Involved in ATP Synthesis (Type of Phosphorylation)	Maximum Yield of ATP Molecules
Aerobic respiration	<i>Pseudomonas aeruginosa</i>	O ₂	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 34
			Total	38
Anaerobic respiration	<i>Paracoccus denitrificans</i>	NO ₃ ⁻ , SO ₄ ⁻² , Fe ⁺³ , CO ₂ , other inorganics	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 1–32
			Total	5–36
Fermentation	<i>Candida albicans</i>	Organics (usually pyruvate)	EMP glycolysis (SLP) Fermentation	2 0
			Total	2

Microbial fermentation processes have been manipulated by humans and are used extensively in the production of various foods and other commercial products, including pharmaceuticals. Microbial fermentation can also be useful for identifying microbes for diagnostic purposes.

Fermentation by some bacteria, like those in yogurt and other soured food products, and by animals in muscles during oxygen depletion, is lactic acid fermentation. The chemical reaction of lactic acid fermentation is as follows:

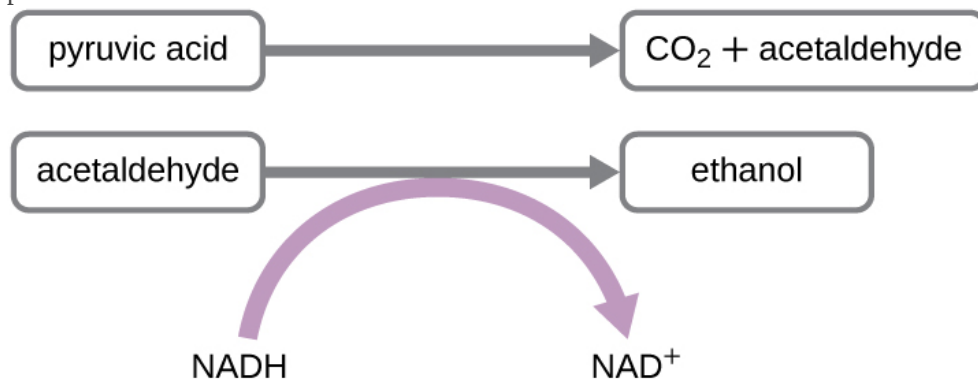
Equation:



Bacteria of several gram-positive genera, including *Lactobacillus*, *Leuconostoc*, and *Streptococcus*, are collectively known as the lactic acid bacteria (LAB), and various strains are important in food production. During yogurt and cheese production, the highly acidic environment generated by lactic acid fermentation denatures proteins contained in milk, causing it to solidify. When lactic acid is the only fermentation product, the process is said to be **homolactic fermentation**; such is the case for *Lactobacillus delbrueckii* and *S. thermophiles* used in yogurt production. However, many bacteria perform **heterolactic fermentation**, producing a mixture of lactic acid, ethanol and/or acetic acid, and CO₂ as a result, because of their use of the branched pentose phosphate pathway instead of the EMP pathway for glycolysis. One important heterolactic fermenter is *Leuconostoc mesenteroides*, which is used for souring vegetables like cucumbers and cabbage, producing pickles and sauerkraut, respectively.

Lactic acid bacteria are also important medically. The production of low pH environments within the body inhibits the establishment and growth of pathogens in these areas. For example, the vaginal microbiota is composed largely of lactic acid bacteria, but when these bacteria are reduced, yeast can proliferate, causing a yeast infection. Additionally, lactic acid bacteria are important in maintaining the health of the gastrointestinal tract and, as such, are the primary component of probiotics.

Another familiar fermentation process is alcohol fermentation, which produces ethanol. The ethanol fermentation reaction is shown in [\[link\]](#). In the first reaction, the enzyme pyruvate decarboxylase removes a carboxyl group from pyruvate, releasing CO₂ gas while producing the two-carbon molecule acetaldehyde. The second reaction, catalyzed by the enzyme alcohol dehydrogenase, transfers an electron from NADH to acetaldehyde, producing ethanol and NAD⁺. The ethanol fermentation of pyruvate by the yeast *Saccharomyces cerevisiae* is used in the production of alcoholic beverages and also makes bread products rise due to CO₂ production. Outside of the food industry, ethanol fermentation of plant products is important in biofuel production.



The chemical reactions of alcohol fermentation are shown here. Ethanol fermentation is important in the production of alcoholic beverages and bread.

Beyond lactic acid fermentation and alcohol fermentation, many other fermentation methods occur in prokaryotes, all for the purpose of ensuring an adequate supply of NAD⁺ for glycolysis ([\[link\]](#)). Without these pathways, glycolysis would not occur and no ATP would be harvested from the breakdown of glucose. It should be noted that most forms of fermentation besides homolactic fermentation produce gas, commonly CO₂ and/or hydrogen gas. Many of these different types of fermentation pathways are also used in food production and each results in the production of different organic acids, contributing to the unique flavor of a particular fermented food product. The propionic acid produced during propionic acid fermentation contributes to the distinctive flavor of Swiss cheese, for example.

Several fermentation products are important commercially outside of the food industry. For example, chemical solvents such as acetone and butanol are produced during acetone-butanol-ethanol fermentation. Complex organic pharmaceutical compounds used in antibiotics (e.g., penicillin), vaccines, and vitamins are produced through mixed acid fermentation. Fermentation products are used in the laboratory to differentiate various bacteria for diagnostic purposes. For example, enteric bacteria are known for their ability to perform mixed acid fermentation, reducing the pH, which can be detected using a pH indicator. Similarly, the bacterial production of acetoin during butanediol fermentation can also be detected. Gas production from fermentation can also be seen in an inverted Durham tube that traps produced gas in a broth culture.

Microbes can also be differentiated according to the substrates they can ferment. For example, *E. coli* can ferment lactose, forming gas, whereas some of its close gram-negative relatives cannot. The ability to ferment the sugar alcohol sorbitol is used to identify the pathogenic enterohemorrhagic O157:H7 strain of *E. coli* because, unlike other *E. coli* strains, it is unable to ferment sorbitol. Last, mannitol fermentation differentiates the mannitol-fermenting *Staphylococcus aureus* from other non-mannitol-fermenting staphylococci.

Common Fermentation Pathways			
Pathway	End Products	Example Microbes	Commercial Products
Acetone-butanol-ethanol	Acetone, butanol, ethanol, CO ₂	<i>Clostridium acetobutylicum</i>	Commercial solvents, gasoline alternative
Alcohol	Ethanol, CO ₂	<i>Candida</i> , <i>Saccharomyces</i>	Beer, bread
Butanediol	Formic and lactic acid; ethanol; acetoin; 2,3 butanediol; CO ₂ ; hydrogen gas	<i>Klebsiella</i> , <i>Enterobacter</i>	Chardonnay wine
Butyric acid	Butyric acid, CO ₂ , hydrogen gas	<i>Clostridium butyricum</i>	Butter
Lactic acid	Lactic acid	<i>Streptococcus</i> , <i>Lactobacillus</i>	Sauerkraut, yogurt, cheese
Mixed acid	Acetic, formic, lactic, and succinic acids; ethanol, CO ₂ , hydrogen gas	<i>Escherichia</i> , <i>Shigella</i>	Vinegar, cosmetics, pharmaceuticals
Propionic acid	Acetic acid, propionic acid, CO ₂	<i>Propionibacterium</i> , <i>Bifidobacterium</i>	Swiss cheese

Note:

- When would a metabolically versatile microbe perform fermentation rather than cellular respiration?

Note:

Identifying Bacteria by Using API Test Panels

Identification of a microbial isolate is essential for the proper diagnosis and appropriate treatment of patients. Scientists have developed techniques that identify bacteria according to their biochemical characteristics. Typically, they either examine the use of specific carbon sources as substrates for fermentation or other metabolic reactions, or they identify fermentation products or specific enzymes present in reactions. In the past, microbiologists have used individual test tubes and plates to conduct biochemical testing. However, scientists, especially those in clinical laboratories, now more frequently use plastic, disposable, multitest panels that contain a number of miniature reaction tubes, each typically including a specific substrate and pH indicator. After inoculation of the test panel with a small sample of the microbe in question and incubation, scientists can compare the results to a database that includes the expected results for specific biochemical reactions for known microbes, thus enabling rapid identification of a sample microbe. These test panels have allowed scientists to reduce costs while improving efficiency and reproducibility by performing a larger number of tests simultaneously.

Many commercial, miniaturized biochemical test panels cover a number of clinically important groups of bacteria and yeasts. One of the earliest and most popular test panels is the Analytical Profile Index (API) panel

invented in the 1970s. Once some basic laboratory characterization of a given strain has been performed, such as determining the strain's Gram morphology, an appropriate test strip that contains 10 to 20 different biochemical tests for differentiating strains within that microbial group can be used. Currently, the various API strips can be used to quickly and easily identify more than 600 species of bacteria, both aerobic and anaerobic, and approximately 100 different types of yeasts. Based on the colors of the reactions when metabolic end products are present, due to the presence of pH indicators, a metabolic profile is created from the results ([link](#)). Microbiologists can then compare the sample's profile to the database to identify the specific microbe.



The API 20NE test strip is used to identify specific strains of gram-negative bacteria outside the Enterobacteriaceae. Here is an API 20NE test strip result for *Photobacterium damsela* ssp. *piscicida*.

Note:

Part 2

Many of Hannah's symptoms are consistent with several different infections, including influenza and pneumonia. However, her sluggish reflexes along with her light sensitivity and stiff neck suggest some possible involvement of the central nervous system, perhaps indicating meningitis. Meningitis is an infection of the cerebrospinal fluid (CSF) around the brain and spinal cord that causes inflammation of the meninges, the protective layers covering the brain. Meningitis can be caused by viruses, bacteria, or fungi. Although all forms of meningitis are serious, bacterial meningitis is particularly serious. Bacterial meningitis may be caused by several different bacteria, but the bacterium *Neisseria meningitidis*, a gram-negative, bean-shaped diplococcus, is a common cause and leads to death within 1 to 2 days in 5% to 10% of patients.

Given the potential seriousness of Hannah's conditions, her physician advised her parents to take her to the hospital in the Gambian capital of Banjul and there have her tested and treated for possible meningitis. After a 3-hour drive to the hospital, Hannah was immediately admitted. Physicians took a blood sample and performed a lumbar puncture to test her CSF. They also immediately started her on a course of the antibiotic ceftriaxone, the drug of choice for treatment of meningitis caused by *N. meningitidis*, without waiting for laboratory test results.

- How might biochemical testing be used to confirm the identity of *N. meningitidis*?
- Why did Hannah's doctors decide to administer antibiotics without waiting for the test results?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Fermentation uses an organic molecule as a final electron acceptor to regenerate NAD^+ from NADH so that glycolysis can continue.
- Fermentation does not involve an electron transport system, and no ATP is made by the fermentation process directly. Fermenters make very little ATP—only two ATP molecules per glucose molecule during glycolysis.
- Microbial fermentation processes have been used for the production of foods and pharmaceuticals, and for the identification of microbes.
- During lactic acid fermentation, pyruvate accepts electrons from NADH and is reduced to lactic acid. Microbes performing **homolactic fermentation** produce only lactic acid as the fermentation product;

microbes performing **heterolactic fermentation** produce a mixture of lactic acid, ethanol and/or acetic acid, and CO₂.

- Lactic acid production by the normal microbiota prevents growth of pathogens in certain body regions and is important for the health of the gastrointestinal tract.
- During ethanol fermentation, pyruvate is first decarboxylated (releasing CO₂) to acetaldehyde, which then accepts electrons from NADH, reducing acetaldehyde to ethanol. Ethanol fermentation is used for the production of alcoholic beverages, for making bread products rise, and for biofuel production.
- Fermentation products of pathways (e.g., propionic acid fermentation) provide distinctive flavors to food products. Fermentation is used to produce chemical solvents (acetone-butanol-ethanol fermentation) and pharmaceuticals (mixed acid fermentation).
- Specific types of microbes may be distinguished by their fermentation pathways and products. Microbes may also be differentiated according to the substrates they are able to ferment.

Multiple Choice

Exercise:

Problem: Which of the following is the purpose of fermentation?

- A. to make ATP
- B. to make carbon molecule intermediates for anabolism
- C. to make NADH
- D. to make NAD⁺

Solution:

D

Exercise:

Problem: Which molecule typically serves as the final electron acceptor during fermentation?

- A. oxygen
- B. NAD⁺
- C. pyruvate
- D. CO₂

Solution:

C

Exercise:

Problem: Which fermentation product is important for making bread rise?

- A. ethanol
- B. CO₂
- C. lactic acid
- D. hydrogen gas

Solution:

B

Exercise:

Problem: Which of the following is not a commercially important fermentation product?

- A. ethanol
- B. pyruvate
- C. butanol
- D. penicillin

Solution:

B

Fill in the Blank

Exercise:

Problem:

The microbe responsible for ethanol fermentation for the purpose of producing alcoholic beverages is _____.

Solution:

yeast (*Saccharomyces cerevisiae*)

Exercise:

Problem:

_____ results in the production of a mixture of fermentation products, including lactic acid, ethanol and/or acetic acid, and CO₂.

Solution:

Heterolactic fermentation

Exercise:

Problem: Fermenting organisms make ATP through the process of _____.

Solution:

glycolysis

Matching

Exercise:

Problem: Match the fermentation pathway with the correct commercial product it is used to produce:

___acetone-butanol-ethanol fermentation	a. bread
___alcohol fermentation	b. pharmaceuticals
___lactic acid fermentation	c. Swiss cheese
___mixed acid fermentation	d. yogurt
___propionic acid fermentation	e. industrial solvents

Solution:

1. e; 2. a; 3. d; 4. b; 5. c

Short Answer

Exercise:

Problem:

Why are some microbes, including *Streptococcus* spp., unable to perform aerobic respiration, even in the presence of oxygen?

Exercise:

Problem: How can fermentation be used to differentiate various types of microbes?

Critical Thinking

Exercise:

Problem:

The bacterium *E. coli* is capable of performing aerobic respiration, anaerobic respiration, and fermentation. When would it perform each process and why? How is ATP made in each case?

Catabolism of Lipids and Proteins

LEARNING OBJECTIVES

- Describe how lipids are catabolized
- Describe how lipid catabolism can be used to identify microbes
- Describe how proteins are catabolized
- Describe how protein catabolism can be used to identify bacteria

Previous sections have discussed the catabolism of glucose, which provides energy to living cells, as well as how polysaccharides like glycogen, starch, and cellulose are degraded to glucose monomers. But microbes consume more than just carbohydrates for food. In fact, the microbial world is known for its ability to degrade a wide range of molecules, both naturally occurring and those made by human processes, for use as carbon sources. In this section, we will see that the pathways for both lipid and protein catabolism connect to those used for carbohydrate catabolism, eventually leading into glycolysis, the transition reaction, and the Krebs cycle pathways. Metabolic pathways should be considered to be porous—that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways.

Lipid Catabolism

Triglycerides are a form of long-term energy storage in animals. They are made of glycerol and three fatty acids (see [\[link\]](#)). Phospholipids compose

the cell and organelle membranes of all organisms except the archaea. Phospholipid structure is similar to triglycerides except that one of the fatty acids is replaced by a phosphorylated head group (see [\[link\]](#)). Triglycerides and phospholipids are broken down first by releasing fatty acid chains (and/or the phosphorylated head group, in the case of phospholipids) from the three-carbon glycerol backbone. The reactions breaking down triglycerides are catalyzed by **lipases** and those involving phospholipids are catalyzed by **phospholipases**. These enzymes contribute to the virulence of certain microbes, such as the bacterium *Staphylococcus aureus* and the fungus *Cryptococcus neoformans*. These microbes use phospholipases to destroy lipids and phospholipids in host cells and then use the catabolic products for energy (see [Virulence Factors of Bacterial and Viral Pathogens](#)).

The resulting products of lipid catabolism, glycerol and fatty acids, can be further degraded. Glycerol can be phosphorylated to glycerol-3-phosphate and easily converted to glyceraldehyde 3-phosphate, which continues through glycolysis. The released fatty acids are catabolized in a process called **β -oxidation**, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains, reducing NAD^+ and FAD to produce NADH and FADH_2 , respectively, whose electrons can be used to make ATP by oxidative phosphorylation. The acetyl groups produced during β -oxidation are carried by coenzyme A to the Krebs cycle, and their movement through this cycle results in their degradation to CO_2 , producing ATP by substrate-level phosphorylation and additional NADH and FADH_2 molecules (see [Appendix C](#) for a detailed illustration of β -oxidation).

Other types of lipids can also be degraded by certain microbes. For example, the ability of certain pathogens, like *Mycobacterium tuberculosis*, to degrade cholesterol contributes to their virulence. The side chains of cholesterol can be easily removed enzymatically, but degradation of the remaining fused rings is more problematic. The four fused rings are sequentially broken in a multistep process facilitated by specific enzymes, and the resulting products, including pyruvate, can be further catabolized in the Krebs cycle.

Note:

- How can lipases and phospholipases contribute to virulence in microbes?

Protein Catabolism

Proteins are degraded through the concerted action of a variety of microbial **protease** enzymes. Extracellular proteases cut proteins internally at specific amino acid sequences, breaking them down into smaller peptides that can then be taken up by cells. Some clinically important pathogens can be identified by their ability to produce a specific type of extracellular protease. For example, the production of the extracellular protease gelatinase by members of the genera *Proteus* and *Serratia* can be used to distinguish them from other gram-negative enteric bacteria. Following inoculation and growth of microbes in gelatin broth, degradation of the gelatin protein due to gelatinase production prevents solidification of gelatin when refrigerated. Other pathogens can be distinguished by their ability to degrade casein, the main protein found in milk. When grown on skim milk agar, production of the extracellular protease caseinase causes degradation of casein, which appears as a zone of clearing around the microbial growth. Caseinase production by the opportunist pathogen *Pseudomonas aeruginosa* can be used to distinguish it from other related gram-negative bacteria.

After extracellular protease degradation and uptake of peptides in the cell, the peptides can then be broken down further into individual amino acids by additional intracellular proteases, and each amino acid can be enzymatically deaminated to remove the amino group. The remaining molecules can then enter the transition reaction or the Krebs cycle.

Note:

- How can protein catabolism help identify microbes?

Note:

Part 3

Because bacterial meningitis progresses so rapidly, Hannah's doctors had decided to treat her aggressively with antibiotics, based on empirical observation of her symptoms. However, laboratory testing to confirm the cause of Hannah's meningitis was still important for several reasons. *N. meningitidis* is an infectious pathogen that can be spread from person to person through close contact; therefore, if tests confirm *N. meningitidis* as the cause of Hannah's symptoms, Hannah's parents and others who came into close contact with her might need to be vaccinated or receive prophylactic antibiotics to lower their risk of contracting the disease. On the other hand, if it turns out that *N. meningitidis* is not the cause, Hannah's doctors might need to change her treatment.

The clinical laboratory performed a Gram stain on Hannah's blood and CSF samples. The Gram stain showed the presence of a bean-shaped gram-negative diplococcus. The technician in the hospital lab cultured Hannah's blood sample on both blood agar and chocolate agar, and the bacterium that grew on both media formed gray, nonhemolytic colonies. Next, he performed an oxidase test on this bacterium and determined that it was oxidase positive. Last, he examined the repertoire of sugars that the bacterium could use as a carbon source and found that the bacterium was positive for glucose and maltose use but negative for lactose and sucrose use. All of these test results are consistent with characteristics of *N. meningitidis*.

- What do these test results tell us about the metabolic pathways of *N. meningitidis*?
- Why do you think that the hospital used these biochemical tests for identification in lieu of molecular analysis by DNA testing?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Collectively, microbes have the ability to degrade a wide variety of carbon sources besides carbohydrates, including lipids and proteins. The catabolic pathways for all of these molecules eventually connect into glycolysis and the Krebs cycle.
- Several types of lipids can be microbially degraded. Triglycerides are degraded by extracellular **lipases**, releasing fatty acids from the glycerol backbone. Phospholipids are degraded by **phospholipases**, releasing fatty acids and the phosphorylated head group from the glycerol backbone. Lipases and phospholipases act as virulence factors for certain pathogenic microbes.
- Fatty acids can be further degraded inside the cell through **β -oxidation**, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains.
- Protein degradation involves extracellular **proteases** that degrade large proteins into smaller peptides. Detection of the extracellular proteases gelatinase and caseinase can be used to differentiate clinically relevant bacteria.

Multiple Choice

Exercise:

Problem:

Which of the following molecules is not produced during the breakdown of phospholipids?

- A. glucose
- B. glycerol
- C. acetyl groups
- D. fatty acids

Solution:

A

Exercise:

Problem: Caseinase is which type of enzyme?

- A. phospholipase
- B. lipase
- C. extracellular protease
- D. intracellular protease

Solution:

C

Exercise:

Problem:

Which of the following is the first step in triglyceride degradation?

- A. removal of fatty acids
- B. β -oxidation
- C. breakage of fused rings
- D. formation of smaller peptides

Solution:

A

Fill in the Blank**Exercise:**

Problem:

The process by which two-carbon units are sequentially removed from fatty acids, producing acetyl-CoA, FADH_2 , and NADH is called

_____.

Solution:

β -oxidation

Exercise:**Problem:**

The NADH and FADH₂ produced during β -oxidation are used to make _____.

Solution:

ATP by oxidative phosphorylation

Exercise:**Problem:**

_____ is a type of medium used to detect the production of an extracellular protease called caseinase.

Solution:

Skim milk agar

Short Answer**Exercise:****Problem:**

How are the products of lipid and protein degradation connected to glucose metabolism pathways?

Exercise:

Problem:

What is the general strategy used by microbes for the degradation of macromolecules?

Critical Thinking**Exercise:****Problem:**

Do you think that β -oxidation can occur in an organism incapable of cellular respiration? Why or why not?

Photosynthesis

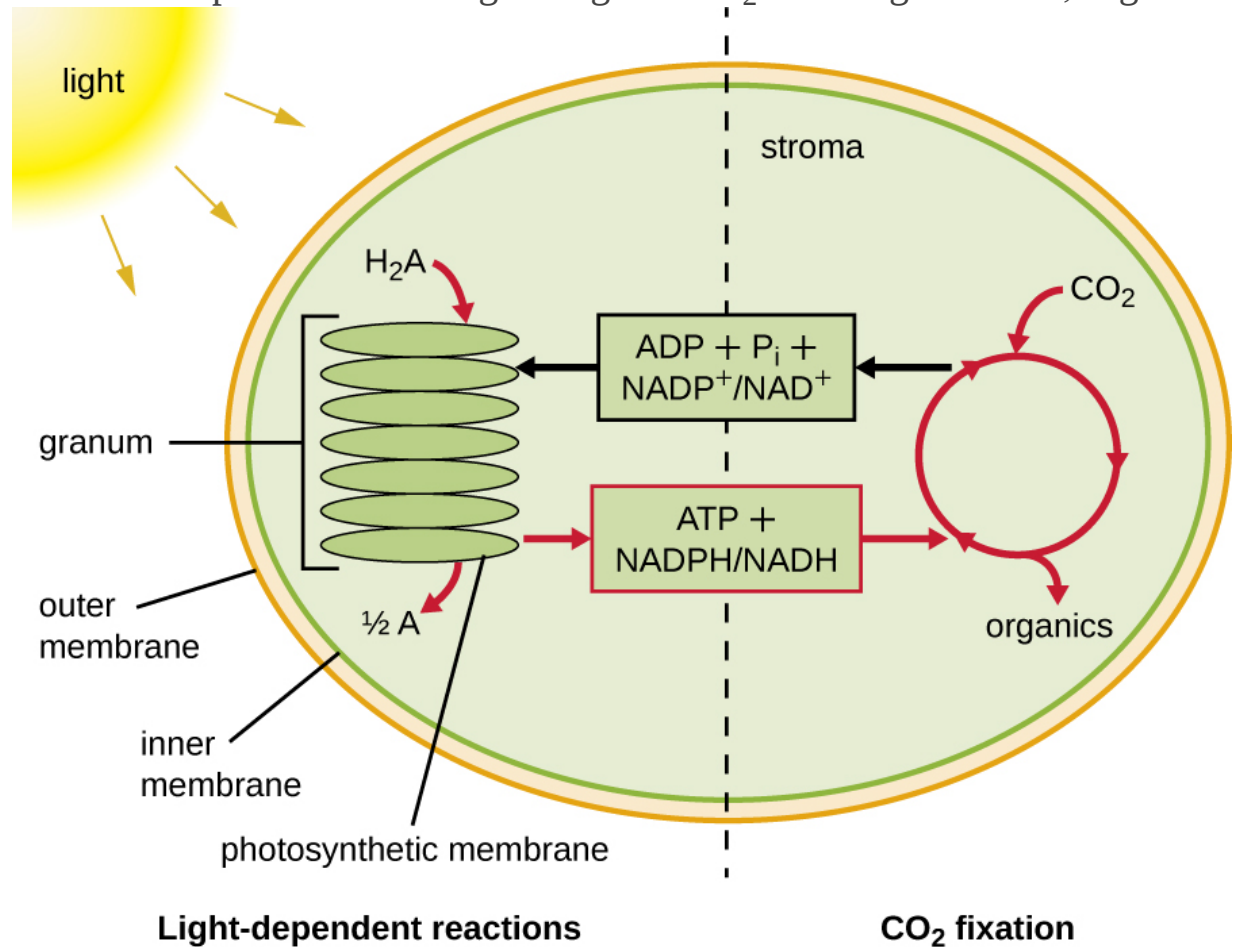
LEARNING OBJECTIVES

- Describe the function and locations of photosynthetic pigments in eukaryotes and prokaryotes
- Describe the major products of the light-dependent and light-independent reactions
- Describe the reactions that produce glucose in a photosynthetic cell
- Compare and contrast cyclic and noncyclic photophosphorylation

Heterotrophic organisms ranging from *E. coli* to humans rely on the chemical energy found mainly in carbohydrate molecules. Many of these carbohydrates are produced by **photosynthesis**, the biochemical process by which phototrophic organisms convert solar energy (sunlight) into chemical energy. Although photosynthesis is most commonly associated with plants, microbial photosynthesis is also a significant supplier of chemical energy, fueling many diverse ecosystems. In this section, we will focus on microbial photosynthesis.

Photosynthesis takes place in two sequential stages: the light-dependent reactions and the light-independent reactions ([\[link\]](#)). In the **light-dependent reactions**, energy from sunlight is absorbed by pigment molecules in photosynthetic membranes and converted into stored chemical energy. In the **light-independent reactions**, the chemical energy produced by the light-dependent reactions is used to drive the assembly of sugar molecules using CO₂; however, these reactions are still light dependent

because the products of the light-dependent reactions necessary for driving them are short-lived. The light-dependent reactions produce ATP and either NADPH or NADH to temporarily store energy. These energy carriers are used in the light-independent reactions to drive the energetically unfavorable process of “fixing” inorganic CO_2 in an organic form, sugar.



The light-dependent reactions of photosynthesis (left) convert light energy into chemical energy, forming ATP and NADPH. These products are used by the light-independent reactions to fix CO_2 , producing organic carbon molecules.

Photosynthetic Structures in Eukaryotes and Prokaryotes

In all phototrophic eukaryotes, photosynthesis takes place inside a **chloroplast**, an organelle that arose in eukaryotes by endosymbiosis of a photosynthetic bacterium (see [Unique Characteristics of Eukaryotic Cells](#)). These chloroplasts are enclosed by a double membrane with inner and outer layers. Within the chloroplast is a third membrane that forms stacked, disc-shaped photosynthetic structures called thylakoids ([link](#)). A stack of thylakoids is called a granum, and the space surrounding the granum within the chloroplast is called stroma.

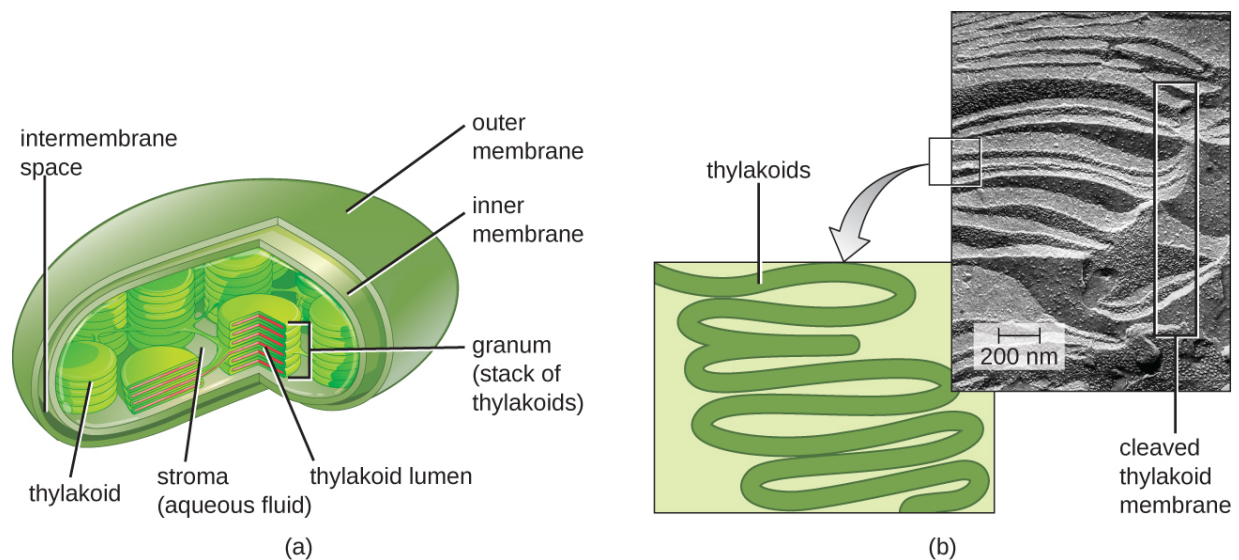
Photosynthetic membranes in prokaryotes, by contrast, are not organized into distinct membrane-enclosed organelles; rather, they are infolded regions of the plasma membrane. In cyanobacteria, for example, these infolded regions are also referred to as thylakoids. In either case, embedded within the thylakoid membranes or other photosynthetic bacterial membranes are **photosynthetic pigment** molecules organized into one or more photosystems, where light energy is actually converted into chemical energy.

Photosynthetic pigments within the photosynthetic membranes are organized into **photosystems**, each of which is composed of a light-harvesting (antennae) complex and a reaction center. The **light-harvesting complex** consists of multiple proteins and associated pigments that each may absorb light energy and, thus, become excited. This energy is transferred from one pigment molecule to another until eventually (after about a millionth of a second) it is delivered to the reaction center. Up to this point, only energy—not electrons—has been transferred between molecules. The **reaction center** contains a pigment molecule that can undergo oxidation upon excitation, actually giving up an electron. It is at this step in photosynthesis that light energy is converted into an excited electron.

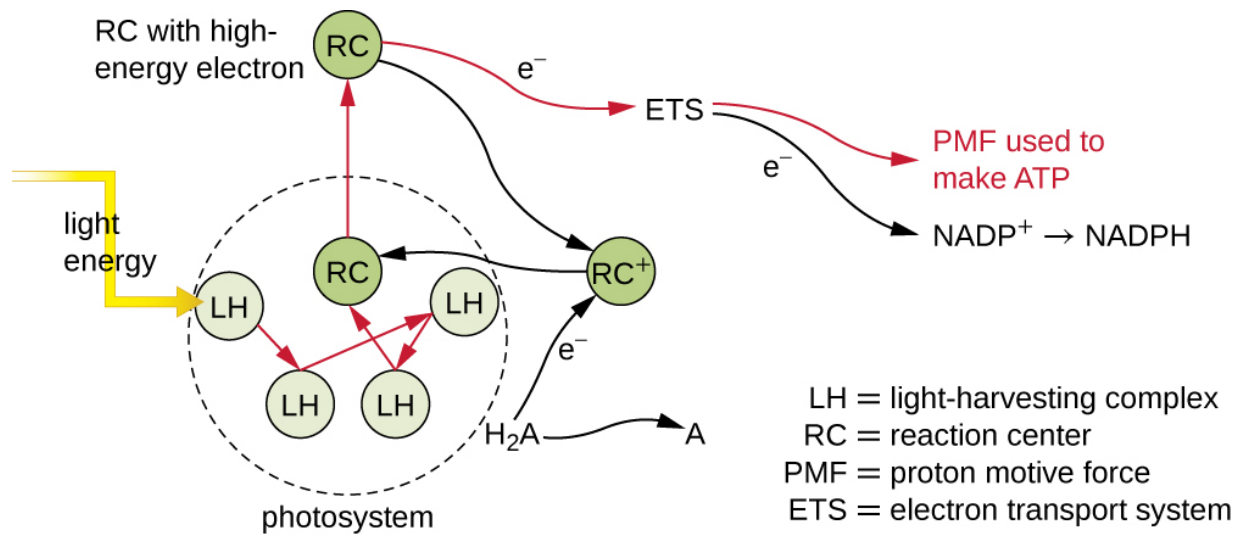
Different kinds of light-harvesting pigments absorb unique patterns of wavelengths (colors) of visible light. Pigments reflect or transmit the wavelengths they cannot absorb, making them appear the corresponding color. Examples of photosynthetic pigments (molecules used to absorb solar energy) are bacteriochlorophylls (green, purple, or red), carotenoids (orange, red, or yellow), chlorophylls (green), phycocyanins (blue), and

phycoerythrins (red). By having mixtures of pigments, an organism can absorb energy from more wavelengths. Because photosynthetic bacteria commonly grow in competition for sunlight, each type of photosynthetic bacteria is optimized for harvesting the wavelengths of light to which it is commonly exposed, leading to stratification of microbial communities in aquatic and soil ecosystems by light quality and penetration.

Once the light harvesting complex transfers the energy to the reaction center, the reaction center delivers its high-energy electrons, one by one, to an electron carrier in an electron transport system, and electron transfer through the ETS is initiated. The ETS is similar to that used in cellular respiration and is embedded within the photosynthetic membrane. Ultimately, the electron is used to produce NADH or NADPH. The electrochemical gradient that forms across the photosynthetic membrane is used to generate ATP by chemiosmosis through the process of photophosphorylation, another example of oxidative phosphorylation ([\[link\]](#)).



(a) Photosynthesis in eukaryotes takes place in chloroplasts, which contain thylakoids stacked into grana. (b) A photosynthetic prokaryote has infolded regions of the plasma membrane that function like thylakoids. (credit: scale bar data from Matt Russell.)



This figure summarizes how a photosystem works. Light harvesting (LH) pigments absorb light energy, converting it to chemical energy. The energy is passed from one LH pigment to another until it reaches a reaction center (RC) pigment, exciting an electron. This high-energy electron is lost from the RC pigment and passed through an electron transport system (ETS), ultimately producing NADH or NADPH and ATP. A reduced molecule (H_2A) donates an electron, replacing electrons to the electron-deficient RC pigment.

Note:

- In a phototrophic eukaryote, where does photosynthesis take place?

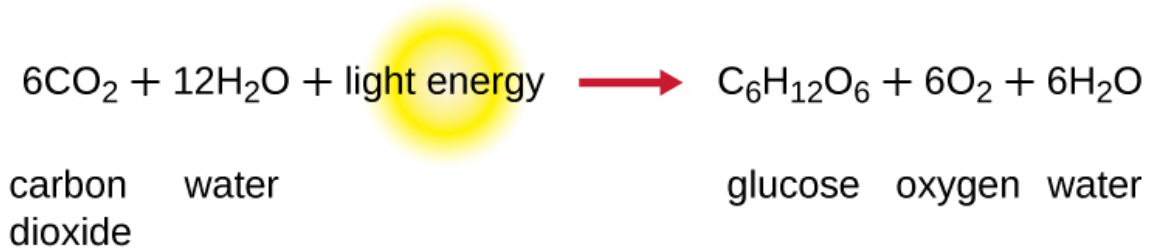
Oxygenic and Anoxygenic Photosynthesis

For photosynthesis to continue, the electron lost from the reaction center pigment must be replaced. The source of this electron (H_2A) differentiates the **oxygenic photosynthesis** of plants and cyanobacteria from **anoxygenic photosynthesis** carried out by other types of bacterial phototrophs ([\[link\]](#)). In oxygenic photosynthesis, H_2O is split and supplies the electron to the reaction center. Because oxygen is generated as a byproduct and is released, this type of photosynthesis is referred to as oxygenic photosynthesis. However, when other reduced compounds serve as the electron donor, oxygen is not generated; these types of photosynthesis are called anoxygenic photosynthesis. Hydrogen sulfide (H_2S) or thiosulfate ($S_2O_3^{2-}$) can serve as the electron donor, generating elemental sulfur and sulfate (SO_4^{2-}) ions, respectively, as a result.

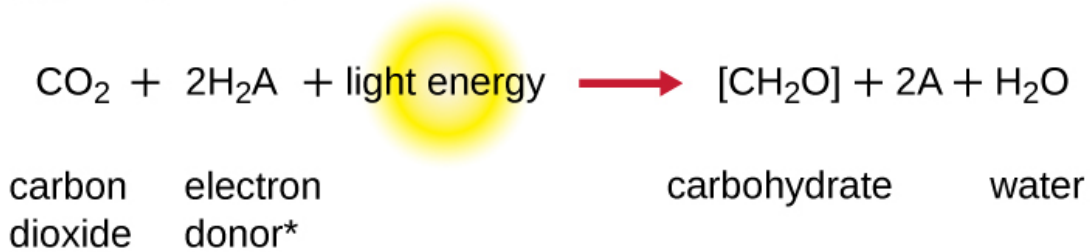
Photosystems have been classified into two types: photosystem I (PSI) and photosystem II (PSII) ([\[link\]](#)). Cyanobacteria and plant chloroplasts have both photosystems, whereas anoxygenic photosynthetic bacteria use only one of the photosystems. Both photosystems are excited by light energy simultaneously. If the cell requires both ATP and NADPH for biosynthesis, then it will carry out **noncyclic photophosphorylation**. Upon passing of the PSII reaction center electron to the ETS that connects PSII and PSI, the lost electron from the PSII reaction center is replaced by the splitting of water. The excited PSI reaction center electron is used to reduce $NADP^+$ to NADPH and is replaced by the electron exiting the ETS. The flow of electrons in this way is called the **Z-scheme**.

If a cell's need for ATP is significantly greater than its need for NADPH, it may bypass the production of reducing power through **cyclic photophosphorylation**. Only PSI is used during cyclic photophosphorylation; the high-energy electron of the PSI reaction center is passed to an ETS carrier and then ultimately returns to the oxidized PSI reaction center pigment, thereby reducing it.

Oxygenic photosynthesis

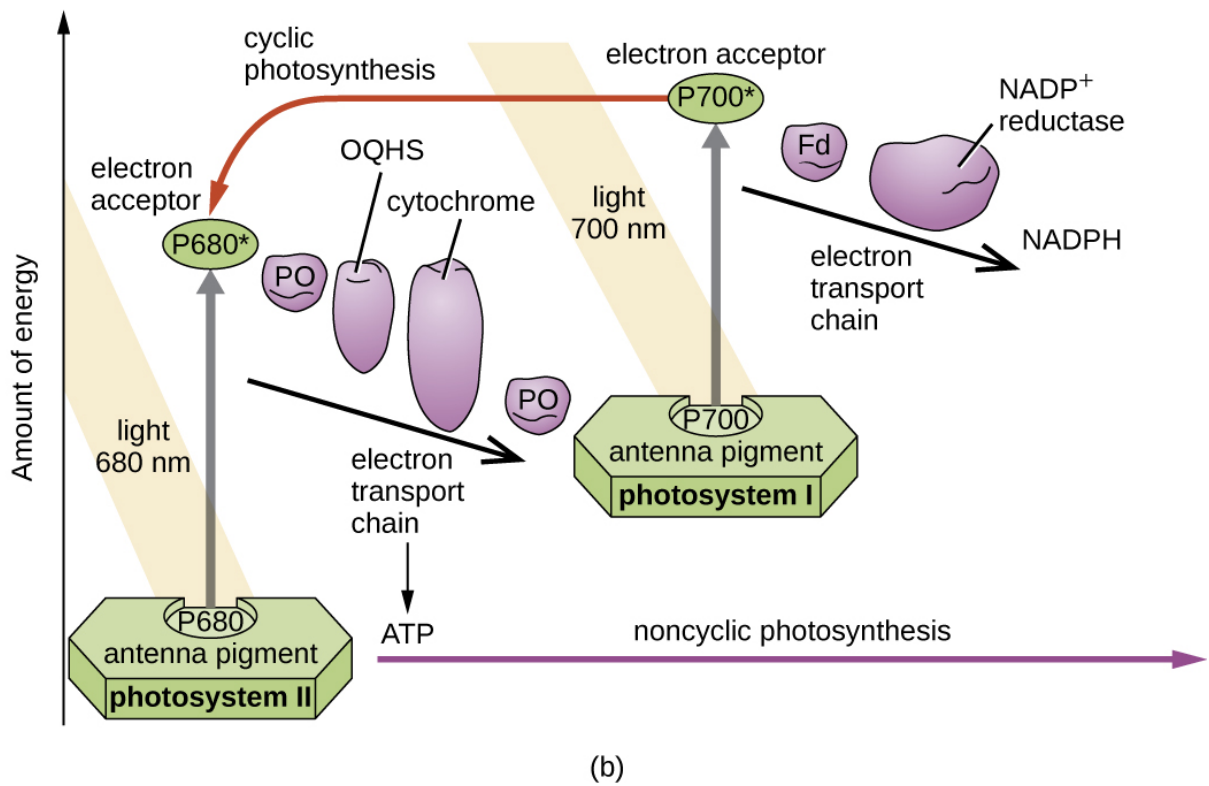
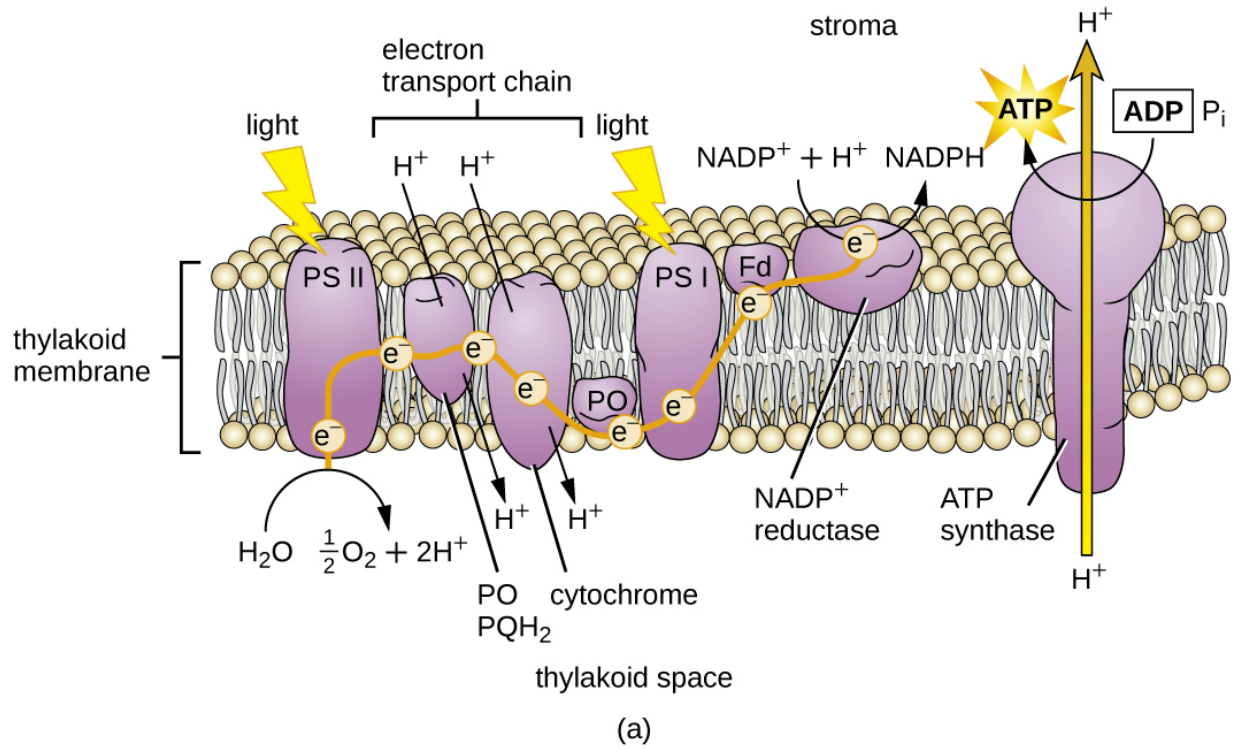


Anoxygenic photosynthesis



*H₂A = H₂O, H₂S, H₂, or other electron donor

Eukaryotes and cyanobacteria carry out oxygenic photosynthesis, producing oxygen, whereas other bacteria carry out anoxygenic photosynthesis, which does not produce oxygen.



(a) PSI and PSII are found on the thylakoid membrane. The high-

energy electron from PSII is passed to an ETS, which generates a proton motive force for ATP synthesis by chemiosmosis, and ultimately replaces the electron lost by the PSI reaction center. The PSI reaction center electron is used to make NADPH. (b) When both ATP and NADPH are required, noncyclic photophosphorylation (in cyanobacteria and plants) provides both. The electron flow described here is referred to as the Z-scheme (shown in yellow in [a]). When the cell's ATP needs outweigh those for NADPH, cyanobacteria and plants will use only PSI, and its reaction center electron is passed to the ETS to generate a proton motive force used for ATP synthesis.

Note:

- Why would a photosynthetic bacterium have different pigments?

Light-Independent Reactions

After the energy from the sun is converted into chemical energy and temporarily stored in ATP and NADPH molecules (having lifespans of millionths of a second), photoautotrophs have the fuel needed to build multicarbon carbohydrate molecules, which can survive for hundreds of millions of years, for long-term energy storage. The carbon comes from CO₂, the gas that is a waste product of cellular respiration.

The **Calvin-Benson cycle** (named for Melvin Calvin [1911–1997] and Andrew Benson [1917–2015]), the biochemical pathway used for fixation of CO₂, is located within the cytoplasm of photosynthetic bacteria and in the stroma of eukaryotic chloroplasts. The light-independent reactions of the Calvin cycle can be organized into three basic stages: fixation, reduction, and regeneration (see [Appendix C](#) for a detailed illustration of the Calvin cycle).

- **Fixation:** The enzyme **ribulose biphosphate carboxylase (RuBisCO)** catalyzes the addition of a CO_2 to ribulose biphosphate (RuBP). This results in the production of 3-phosphoglycerate (3-PGA).
- **Reduction:** Six molecules of both ATP and NADPH (from the light-dependent reactions) are used to convert 3-PGA into glyceraldehyde 3-phosphate (G3P). Some G3P is then used to build glucose.
- **Regeneration:** The remaining G3P not used to synthesize glucose is used to regenerate RuBP, enabling the system to continue CO_2 fixation. Three more molecules of ATP are used in these regeneration reactions.

The Calvin cycle is used extensively by plants and photoautotrophic bacteria, and the enzyme RuBisCO is said to be the most plentiful enzyme on earth, composing 30%–50% of the total soluble protein in plant chloroplasts.[\[footnote\]](#) However, besides its prevalent use in photoautotrophs, the Calvin cycle is also used by many nonphotosynthetic chemoautotrophs to fix CO_2 . Additionally, other bacteria and archaea use alternative systems for CO_2 fixation. Although most bacteria using Calvin cycle alternatives are chemoautotrophic, certain green sulfur photoautotrophic bacteria have been also shown to use an alternative CO_2 fixation pathway.

A. Dhingra et al. “Enhanced Translation of a Chloroplast-Expressed *RbcS* Gene Restores Small Subunit Levels and Photosynthesis in Nuclear *RbcS* Antisense Plants.” *Proceedings of the National Academy of Sciences of the United States of America* 101 no. 16 (2004):6315–6320.

Note:

- Describe the three stages of the Calvin cycle.

Key Concepts and Summary

- Heterotrophs depend on the carbohydrates produced by autotrophs, many of which are photosynthetic, converting solar energy into chemical energy.
- Different photosynthetic organisms use different mixtures of **photosynthetic pigments**, which increase the range of the wavelengths of light an organism can absorb.
- **Photosystems** (PSI and PSII) each contain a **light-harvesting complex**, composed of multiple proteins and associated pigments that absorb light energy. The **light-dependent reactions** of photosynthesis convert solar energy into chemical energy, producing ATP and NADPH or NADH to temporarily store this energy.
- In **oxygenic photosynthesis**, H₂O serves as the electron donor to replace the reaction center electron, and oxygen is formed as a byproduct. In **anoxygenic photosynthesis**, other reduced molecules like H₂S or thiosulfate may be used as the electron donor; as such, oxygen is not formed as a byproduct.
- **Noncyclic photophosphorylation** is used in oxygenic photosynthesis when there is a need for both ATP and NADPH production. If a cell's needs for ATP outweigh its needs for NADPH, then it may carry out **cyclic photophosphorylation** instead, producing only ATP.
- The **light-independent reactions** of photosynthesis use the ATP and NADPH from the light-dependent reactions to fix CO₂ into organic sugar molecules.

Multiple Choice

Exercise:

Problem:

During the light-dependent reactions, which molecule loses an electron?

- A. a light-harvesting pigment molecule
- B. a reaction center pigment molecule
- C. NADPH
- D. 3-phosphoglycerate

Solution:

B

Exercise:

Problem:

In prokaryotes, in which direction are hydrogen ions pumped by the electron transport system of photosynthetic membranes?

- A. to the outside of the plasma membrane
- B. to the inside (cytoplasm) of the cell
- C. to the stroma
- D. to the intermembrane space of the chloroplast

Solution:

A

Exercise:

Problem:

Which of the following does not occur during cyclic photophosphorylation in cyanobacteria?

- A. electron transport through an ETS
- B. photosystem I use
- C. ATP synthesis
- D. NADPH formation

Solution:

D

Exercise:

Problem:

Which of the following are two products of the light-dependent reactions?

- A. glucose and NADPH
- B. NADPH and ATP
- C. glyceraldehyde 3-phosphate and CO₂
- D. glucose and oxygen

Solution:

B

True/False**Exercise:**

Problem: Photosynthesis always results in the formation of oxygen.

Solution:

False

Fill in the Blank**Exercise:****Problem:**

The enzyme responsible for CO₂ fixation during the Calvin cycle is called _____.

Solution:

ribulose biphosphate carboxylase (RuBisCO)

Exercise:

Problem:

The types of pigment molecules found in plants, algae, and cyanobacteria are _____ and _____.

Solution:

chlorophylls and carotenoids

Short Answer

Exercise:

Problem:

Why would an organism perform cyclic phosphorylation instead of noncyclic phosphorylation?

Exercise:

Problem:

What is the function of photosynthetic pigments in the light-harvesting complex?

Critical Thinking

Exercise:

Problem:

Is life dependent on the carbon fixation that occurs during the light-independent reactions of photosynthesis? Explain.

Biogeochemical Cycles

LEARNING OBJECTIVES

- Define and describe the importance of microorganisms in the biogeochemical cycles of carbon, nitrogen, and sulfur
- Define and give an example of bioremediation

Energy flows directionally through ecosystems, entering as sunlight for phototrophs or as inorganic molecules for chemoautotrophs. The six most common elements associated with organic molecules—carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur—take a variety of chemical forms and may exist for long periods in the atmosphere, on land, in water, or beneath earth's surface. Geologic processes, such as erosion, water drainage, the movement of the continental plates, and weathering, all are involved in the cycling of elements on earth. Because geology and chemistry have major roles in the study of this process, the recycling of inorganic matter between living organisms and their nonliving environment is called a **biogeochemical cycle**. Here, we will focus on the function of microorganisms in these cycles, which play roles at each step, most frequently interconverting oxidized versions of molecules with reduced ones.

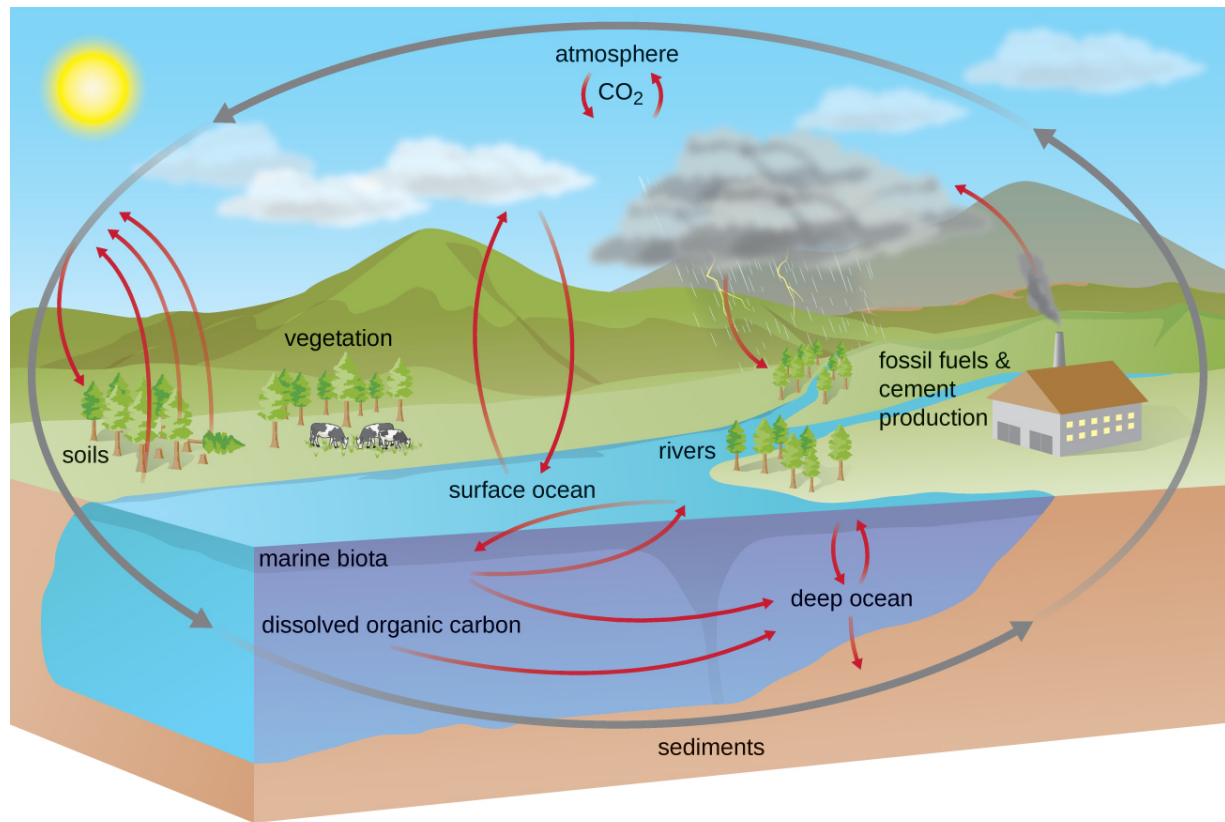
Carbon Cycle

Carbon is one of the most important elements to living organisms, as shown by its abundance and presence in all organic molecules. The carbon cycle exemplifies the connection between organisms in various ecosystems. Carbon is exchanged between heterotrophs and autotrophs within and

between ecosystems primarily by way of atmospheric CO₂, a fully oxidized version of carbon that serves as the basic building block that autotrophs use to build multicarbon, high-energy organic molecules such as glucose. Photoautotrophs and chemoautotrophs harness energy from the sun and from inorganic chemical compounds, respectively, to covalently bond carbon atoms together into reduced organic compounds whose energy can be later accessed through the processes of respiration and fermentation ([link](#)).

Overall, there is a constant exchange of CO₂ between the heterotrophs (which produce CO₂ as a result of respiration or fermentation) and the autotrophs (which use the CO₂ for fixation). Autotrophs also respire or ferment, consuming the organic molecules they form; they do not fix carbon for heterotrophs, but rather use it for their own metabolic needs.

Bacteria and archaea that use methane as their carbon source are called methanotrophs. Reduced one-carbon compounds like methane accumulate in certain anaerobic environments when CO₂ is used as a terminal electron acceptor in anaerobic respiration by archaea called methanogens. Some methanogens also ferment acetate (two carbons) to produce methane and CO₂. Methane accumulation due to methanogenesis occurs in both natural anaerobic soil and aquatic environments; methane accumulation also occurs as a result of animal husbandry because methanogens are members of the normal microbiota of ruminants. Environmental methane accumulation due to methanogenesis is of consequence because it is a strong greenhouse gas, and methanotrophs help to reduce atmospheric methane levels.



Carbon cycle

This figure summarizes the carbon cycle. Eukaryotes participate in aerobic respiration, fermentation, and oxygenic photosynthesis. Prokaryotes participate in all the steps shown. (credit: modification of work by NOAA)

Note:

- Describe the interaction between heterotrophs and autotrophs in the carbon cycle.

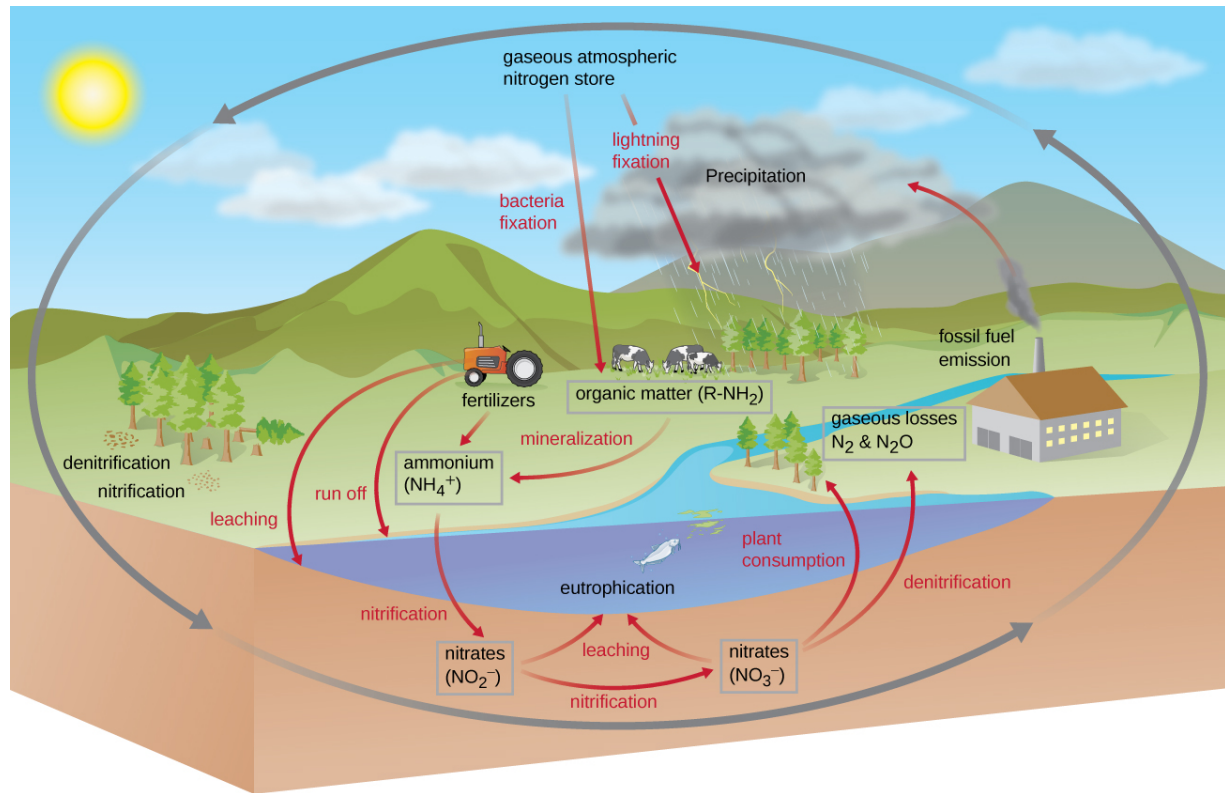
Nitrogen Cycle

Many biological macromolecules, including proteins and nucleic acids, contain nitrogen; however, getting nitrogen into living organisms is difficult. Prokaryotes play essential roles in the nitrogen cycle ([\[link\]](#)), transforming nitrogen between various forms for their own needs, benefiting other organisms indirectly. Plants and phytoplankton cannot incorporate nitrogen from the atmosphere (where it exists as tightly bonded, triple covalent N_2), even though this molecule composes approximately 78% of the atmosphere. Nitrogen enters the living world through free-living and symbiotic bacteria, which incorporate nitrogen into their macromolecules through specialized biochemical pathways called **nitrogen fixation**. Cyanobacteria in aquatic ecosystems fix inorganic nitrogen (from nitrogen gas) into ammonia (NH_3) that can be easily incorporated into biological macromolecules. *Rhizobium* bacteria ([\[link\]](#)) also fix nitrogen and live symbiotically in the root nodules of legumes (such as beans, peanuts, and peas), providing them with needed organic nitrogen while receiving fixed carbon as sugar in exchange. Free-living bacteria, such as members of the genus *Azotobacter*, are also able to fix nitrogen.

The nitrogen that enters living systems by nitrogen fixation is eventually converted from organic nitrogen back into nitrogen gas by microbes through three steps: ammonification, nitrification, and denitrification. In terrestrial systems, the first step is the ammonification process, in which certain bacteria and fungi convert nitrogenous waste from living animals or from the remains of dead organisms into ammonia (NH_3). This ammonia is then oxidized to nitrite (NO_2^-), then to nitrate (NO_3^-), by nitrifying soil bacteria such as members of the genus *Nitrosomonas*, through the process of nitrification. Last, the process of denitrification occurs, whereby soil bacteria, such as members of the genera *Pseudomonas* and *Clostridium*, use nitrate as a terminal electron acceptor in anaerobic respiration, converting it into nitrogen gas that reenters the atmosphere. A similar process occurs in the marine nitrogen cycle, where these three processes are performed by marine bacteria and archaea.

Human activity releases nitrogen into the environment by the use of artificial fertilizers that contain nitrogen and phosphorus compounds, which are then washed into lakes, rivers, and streams by surface runoff. A major effect from fertilizer runoff is saltwater and freshwater eutrophication, in

which nutrient runoff causes the overgrowth and subsequent death of aquatic algae, making water sources anaerobic and inhospitable for the survival of aquatic organisms.



Nitrogen cycle

This figure summarizes the nitrogen cycle. Note that specific groups of prokaryotes each participate in every step in the cycle. (credit: modification of work by NOAA)

Note:

- What are the three steps of the nitrogen cycle?

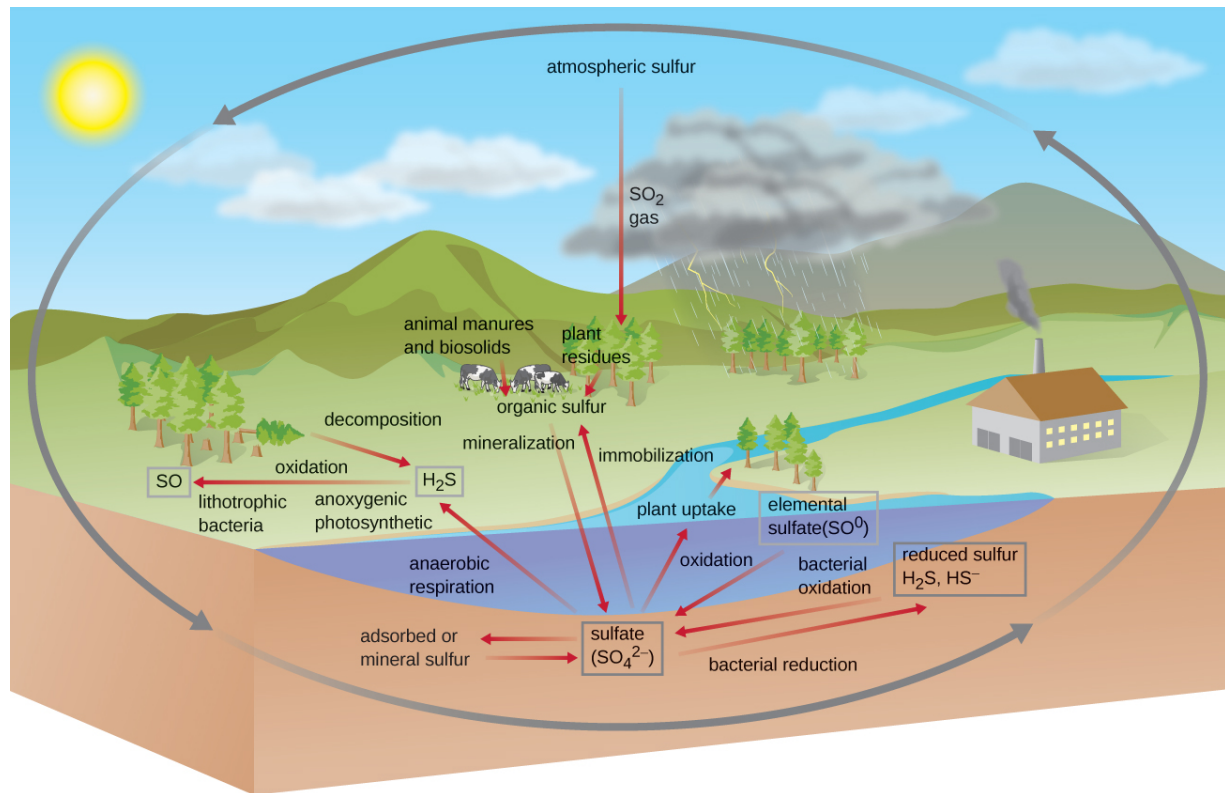
Note:

To learn more about the nitrogen cycle, visit the [PBS](#) website.

Sulfur Cycle

Sulfur is an essential element for the macromolecules of living organisms. As part of the amino acids cysteine and methionine, it is involved in the formation of proteins. It is also found in several vitamins necessary for the synthesis of important biological molecules like coenzyme A. Several groups of microbes are responsible for carrying out processes involved in the sulfur cycle ([link](#)). Anoxygenic photosynthetic bacteria as well as chemoautotrophic archaea and bacteria use hydrogen sulfide as an electron donor, oxidizing it first to elemental sulfur (S^0), then to sulfate (SO_4^{2-}). This leads to stratification of hydrogen sulfide in soil, with levels increasing at deeper, more anaerobic depths.

Many bacteria and plants can use sulfate as a sulfur source. Decomposition of dead organisms by fungi and bacteria remove sulfur groups from amino acids, producing hydrogen sulfide, returning inorganic sulfur to the environment.



Sulfur cycle

This figure summarizes the sulfur cycle. Note that specific groups of prokaryotes each may participate in every step in the cycle. (credit: modification of work by NOAA)

Note:

- Which groups of microbes carry out the sulfur cycle?

Other Biogeochemical Cycles

Beyond their involvement in the carbon, nitrogen, and sulfur cycles, prokaryotes are involved in other biogeochemical cycles as well. Like the carbon, nitrogen, and sulfur cycles, several of these additional biogeochemical cycles, such as the iron (Fe), manganese (Mn), and chromium (Cr) cycles, also involve redox chemistry, with prokaryotes playing roles in both oxidation and reduction. Several other elements undergo chemical cycles that do not involve redox chemistry. Examples of these are phosphorus (P), calcium (Ca), and silica (Si) cycles. The cycling of these elements is particularly important in oceans because large quantities of these elements are incorporated into the exoskeletons of marine organisms. These biogeochemical cycles do not involve redox chemistry but instead involve fluctuations in the solubility of compounds containing calcium, phosphorous, and silica. The overgrowth of naturally occurring microbial communities is typically limited by the availability of nitrogen (as previously mentioned), phosphorus, and iron. Human activities introducing excessive amounts of iron, nitrogen, or phosphorus (typically from detergents) may lead to eutrophication.

Bioremediation

Microbial **bioremediation** leverages microbial metabolism to remove **xenobiotics** or other pollutants. Xenobiotics are compounds synthesized by humans and introduced into the environment in much higher concentrations than would naturally occur. Such environmental contamination may involve adhesives, dyes, flame retardants, lubricants, oil and petroleum products, organic solvents, pesticides, and products of the combustion of gasoline and oil. Many xenobiotics resist breakdown, and some accumulate in the food chain after being consumed or absorbed by fish and wildlife, which, in turn, may be eaten by humans. Of particular concern are contaminants like polycyclic aromatic hydrocarbon (PAH), a carcinogenic xenobiotic found in crude oil, and trichloroethylene (TCE), a common groundwater contaminant.

Bioremediation processes can be categorized as in situ or ex situ. Bioremediation conducted at the site of contamination is called in situ bioremediation and does not involve movement of contaminated material. In contrast, ex situ bioremediation involves the removal of contaminated

material from the original site so that it can be treated elsewhere, typically in a large, lined pit where conditions are optimized for degradation of the contaminant.

Some bioremediation processes rely on microorganisms that are indigenous to the contaminated site or material. Enhanced bioremediation techniques, which may be applied to either in situ or ex situ processing, involve the addition of nutrients and/or air to encourage the growth of pollution-degrading microbes; they may also involve the addition of non-native microbes known for their ability to degrade contaminants. For example, certain bacteria of the genera *Rhodococcus* and *Pseudomonas* are known for their ability to degrade many environmental contaminants, including aromatic compounds like those found in oil, down to CO₂. The genes encoding their degradatory enzymes are commonly found on plasmids. Others, like *Alcanivorax borkumensis*, produce surfactants that are useful in the solubilization of the hydrophobic molecules found in oil, making them more accessible to other microbes for degradation.

Note:

- Compare and contrast the benefits of in situ and ex situ bioremediation.

Note:

Resolution

Although there is a DNA test specific for *Neisseria meningitidis*, it is not practical for use in some developing countries because it requires expensive equipment and a high level of expertise to perform. The hospital in Banjul was not equipped to perform DNA testing. Biochemical testing, however, is much less expensive and is still effective for microbial identification.

Fortunately for Hannah, her symptoms began to resolve with antibiotic therapy. Patients who survive bacterial meningitis often suffer from long-

term complications such as brain damage, hearing loss, and seizures, but after several weeks of recovery, Hannah did not seem to be exhibiting any long-term effects and her behavior returned to normal. Because of her age, her parents were advised to monitor her closely for any signs of developmental issues and have her regularly evaluated by her pediatrician. *N. meningitidis* is found in the normal respiratory microbiota in 10%–20% of the human population.^[footnote] In most cases, it does not cause disease, but for reasons not fully understood, the bacterium can sometimes invade the bloodstream and cause infections in other areas of the body, including the brain. The disease is more common in infants and children, like Hannah.

Centers for Disease Control and Prevention. “Meningococcal Disease: Causes and Transmission.”

<http://www.cdc.gov/meningococcal/about/causes-transmission.html>.

Accessed September 12, 2016.

The prevalence of meningitis caused by *N. meningitidis* is particularly high in the so-called meningitis belt, a region of sub-Saharan African that includes 26 countries stretching from Senegal to Ethiopia (^[link]). The reasons for this high prevalence are not clear, but several factors may contribute to higher rates of transmission, such as the dry, dusty climate; overcrowding and low standards of living; and the relatively low immunocompetence and nutritional status of the population.^[footnote] A vaccine against four bacterial strains of *N. meningitidis* is available. Vaccination is recommended for 11- and 12-year-old children, with a booster at age 16 years. Vaccination is also recommended for young people who live in close quarters with others (e.g., college dormitories, military barracks), where the disease is more easily transmitted. Travelers visiting the “meningitis belt” should also be vaccinated, especially during the dry season (December through June) when the prevalence is highest.^[footnote]
^[footnote]

Centers for Disease Control and Prevention. “Meningococcal Disease in Other Countries.” <http://www.cdc.gov/meningococcal/global.html>.

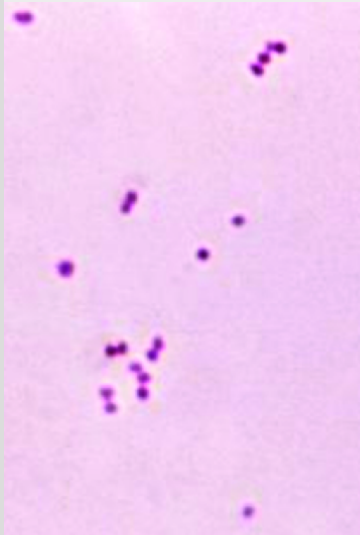
Accessed September 12, 2016.

Centers for Disease Control and Prevention. “Health Information for Travelers to the Gambia: Traveler View.”

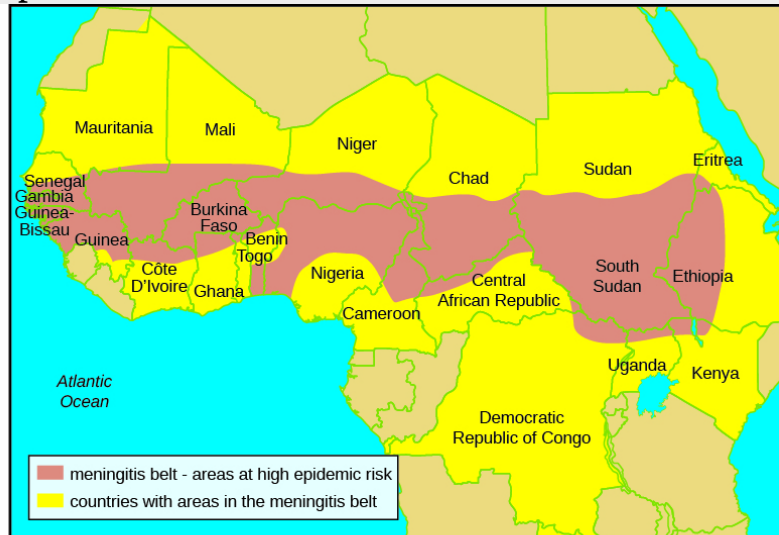
<http://wwwnc.cdc.gov/travel/destinations/traveler/none/the-gambia>.

Accessed September 12, 2016.

Centers for Disease Control and Prevention. “Meningococcal: Who Needs to Be Vaccinated?” <http://www.cdc.gov/vaccines/vpd-vac/mening/who-vaccinate.htm>. Accessed September 12, 2016.



(a)



(b)

(a) *Neisseria meningitidis* is a gram-negative diplococcus, as shown in this gram-stained sample. (b) The “meningitis belt” is the area of sub-Saharan Africa with high prevalence of meningitis caused by *N. meningitidis*. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- The recycling of inorganic matter between living organisms and their nonliving environment is called a **biogeochemical cycle**. Microbes play significant roles in these cycles.
- In the **carbon cycle**, heterotrophs degrade reduced organic molecule to produce carbon dioxide, whereas autotrophs fix carbon dioxide to produce organics. **Methanogens** typically form methane by using CO_2

- as a final electron acceptor during anaerobic respiration; methanotrophs oxidize the methane, using it as their carbon source.
- In the **nitrogen cycle**, nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia (ammonification). The ammonia can then be oxidized to nitrite and nitrate (nitrification). Nitrates can then be assimilated by plants. Soil bacteria convert nitrate back to nitrogen gas (denitrification).
 - In **sulfur cycling**, many anoxygenic photosynthesizers and chemoautotrophs use hydrogen sulfide as an electron donor, producing elemental sulfur and then sulfate; sulfate-reducing bacteria and archaea then use sulfate as a final electron acceptor in anaerobic respiration, converting it back to hydrogen sulfide.
 - Human activities that introduce excessive amounts of naturally limited nutrients (like iron, nitrogen, or phosphorus) to aquatic systems may lead to eutrophication.
 - Microbial **bioremediation** is the use of microbial metabolism to remove or degrade **xenobiotics** and other environmental contaminants and pollutants. Enhanced bioremediation techniques may involve the introduction of non-native microbes specifically chosen or engineered for their ability to degrade contaminants.

Multiple Choice

Exercise:

Problem:

Which of the following is the group of archaea that can use CO_2 as their final electron acceptor during anaerobic respiration, producing CH_4 ?

- A. methylotrophs
- B. methanotrophs
- C. methanogens
- D. anoxygenic photosynthesizers

Solution:

C

Exercise:

Problem:

Which of the following processes is not involved in the conversion of organic nitrogen to nitrogen gas?

- A. nitrogen fixation
- B. ammonification
- C. nitrification
- D. denitrification

Solution:

A

Exercise:

Problem:

Which of the following processes produces hydrogen sulfide?

- A. anoxygenic photosynthesis
- B. oxygenic photosynthesis
- C. anaerobic respiration
- D. chemoautotrophy

Solution:

C

Exercise:

Problem:

The biogeochemical cycle of which of the following elements is based on changes in solubility rather than redox chemistry?

- A. carbon
- B. sulfur
- C. nitrogen
- D. phosphorus

Solution:

D

Fill in the Blank

Exercise:

Problem:

The molecule central to the carbon cycle that is exchanged within and between ecosystems, being produced by heterotrophs and used by autotrophs, is _____.

Solution:

carbon dioxide

Exercise:

Problem:

The use of microbes to remove pollutants from a contaminated system is called _____.

Solution:

bioremediation

True/False

Exercise:

Problem:

There are many naturally occurring microbes that have the ability to degrade several of the compounds found in oil.

Solution:

True

Short Answer**Exercise:****Problem:**

Why must autotrophic organisms also respire or ferment in addition to fixing CO₂?

Exercise:

Problem: How can human activity lead to eutrophication?

Critical Thinking**Exercise:****Problem:**

In considering the symbiotic relationship between *Rhizobium* species and their plant hosts, what metabolic activity does each organism perform that benefits the other member of the pair?

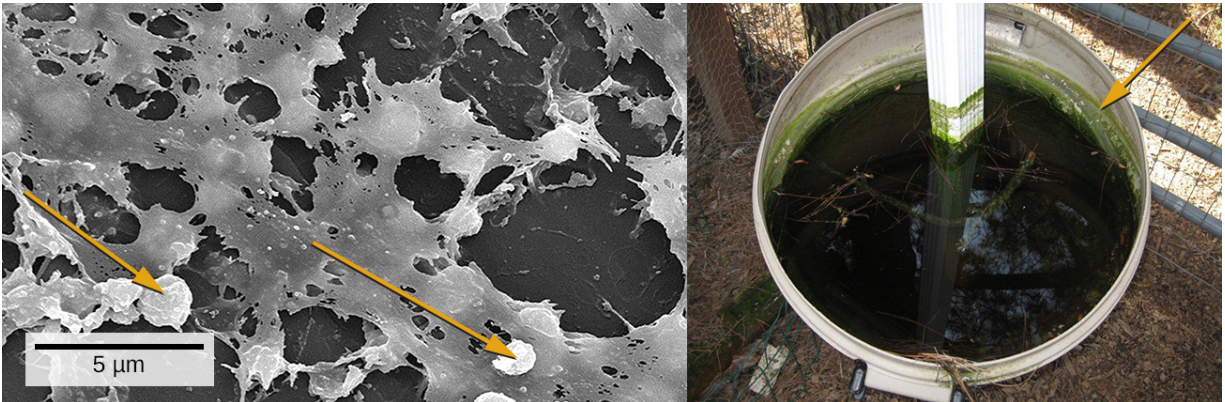
Introduction

class="introduction"

Medical devices that are inserted into a patient's body often become contaminated with a thin biofilm of microorganisms enmeshed in the sticky material they secrete. The electron micrograph (left) shows the inside walls of an in-dwelling catheter.

Arrows point to the round cells of *Staphylococcus aureus* bacteria attached to the layers of extracellular substrate. The garbage can (right) served as a rain collector. The arrow points to a green biofilm

on the sides of
the container.
(credit left:
modification of
work by
Centers for
Disease
Control and
Prevention;
credit right:
modification of
work by
NASA)



We are all familiar with the slimy layer on a pond surface or that makes rocks slippery. These are examples of biofilms—microorganisms embedded in thin layers of matrix material ([\[link\]](#)). Biofilms were long considered random assemblages of cells and had little attention from researchers. Recently, progress in visualization and biochemical methods has revealed that biofilms are an organized ecosystem within which many cells, usually of different species of bacteria, fungi, and algae, interact through cell signaling and coordinated responses. The biofilm provides a protected environment in harsh conditions and aids colonization by microorganisms. Biofilms also have clinical importance. They form on medical devices, resist routine cleaning and sterilization, and cause health-acquired infections. Within the body, biofilms form on the teeth as plaque, in the

lungs of patients with cystic fibrosis, and on the cardiac tissue of patients with endocarditis. The slime layer helps protect the cells from host immune defenses and antibiotic treatments.

Studying biofilms requires new approaches. Because of the cells' adhesion properties, many of the methods for culturing and counting cells that are explored in this chapter are not easily applied to biofilms. This is the beginning of a new era of challenges and rewarding insight into the ways that microorganisms grow and thrive in nature.

How Microbes Grow

LEARNING OBJECTIVES

- Define the generation time for growth based on binary fission
- Identify and describe the activities of microorganisms undergoing typical phases of binary fission (simple cell division) in a growth curve
- Explain several laboratory methods used to determine viable and total cell counts in populations undergoing exponential growth
- Describe examples of cell division not involving binary fission, such as budding or fragmentation
- Describe the formation and characteristics of biofilms
- Identify health risks associated with biofilms and how they are addressed
- Describe quorum sensing and its role in cell-to-cell communication and coordination of cellular activities

Note:

Part 1

Jeni, a 24-year-old pregnant woman in her second trimester, visits a clinic with complaints of high fever, 38.9 °C (102 °F), fatigue, and muscle aches—typical flu-like signs and symptoms. Jeni exercises regularly and follows a nutritious diet with emphasis on organic foods, including raw milk that she purchases from a local farmer's market. All of her immunizations are up to date. However, the health-care provider who sees Jeni is concerned and orders a blood sample to be sent for testing by the microbiology laboratory.

- Why is the health-care provider concerned about Jeni's signs and symptoms?

Jump to the [next](#) Clinical Focus box

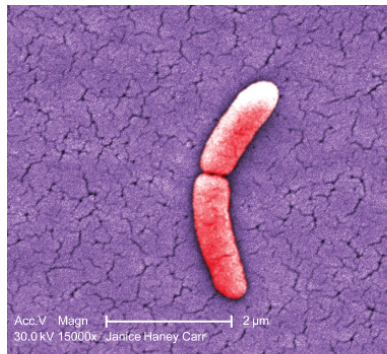
The bacterial cell cycle involves the formation of new cells through the replication of DNA and partitioning of cellular components into two daughter cells. In prokaryotes, reproduction is always asexual, although extensive genetic recombination in the form of horizontal gene transfer takes place, as will be explored in a different chapter. Most bacteria have a single circular chromosome; however, some exceptions exist. For example, *Borrelia burgdorferi*, the causative agent of Lyme disease, has a linear chromosome.

Binary Fission

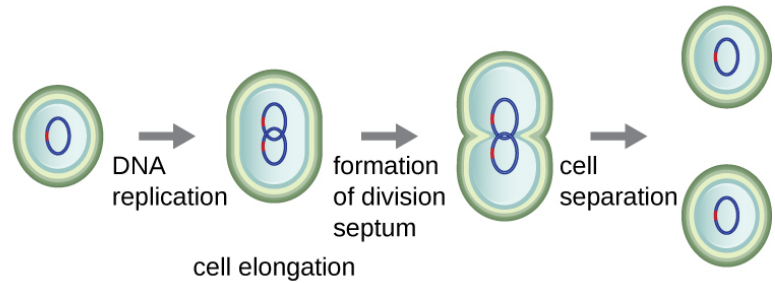
The most common mechanism of cell replication in bacteria is a process called **binary fission**, which is depicted in [\[link\]](#). Before dividing, the cell grows and increases its number of cellular components. Next, the replication of DNA starts at a location on the circular chromosome called the origin of replication, where the chromosome is attached to the inner cell membrane. Replication continues in opposite directions along the chromosome until the terminus is reached.

The center of the enlarged cell constricts until two daughter cells are formed, each offspring receiving a complete copy of the parental genome and a division of the cytoplasm (cytokinesis). This process of cytokinesis and cell division is directed by a protein called FtsZ. FtsZ assembles into a Z ring on the cytoplasmic membrane ([\[link\]](#)). The Z ring is anchored by FtsZ-binding proteins and defines the division plane between the two daughter cells. Additional proteins required for cell division are added to the Z ring to form a structure called the divisome. The divisome activates to produce a peptidoglycan cell wall and build a **septum** that divides the two daughter cells. The daughter cells are separated by the division septum, where all of the cells' outer layers (the cell wall and outer membranes, if

present) must be remodeled to complete division. For example, we know that specific enzymes break bonds between the monomers in peptidoglycans and allow addition of new subunits along the division septum.

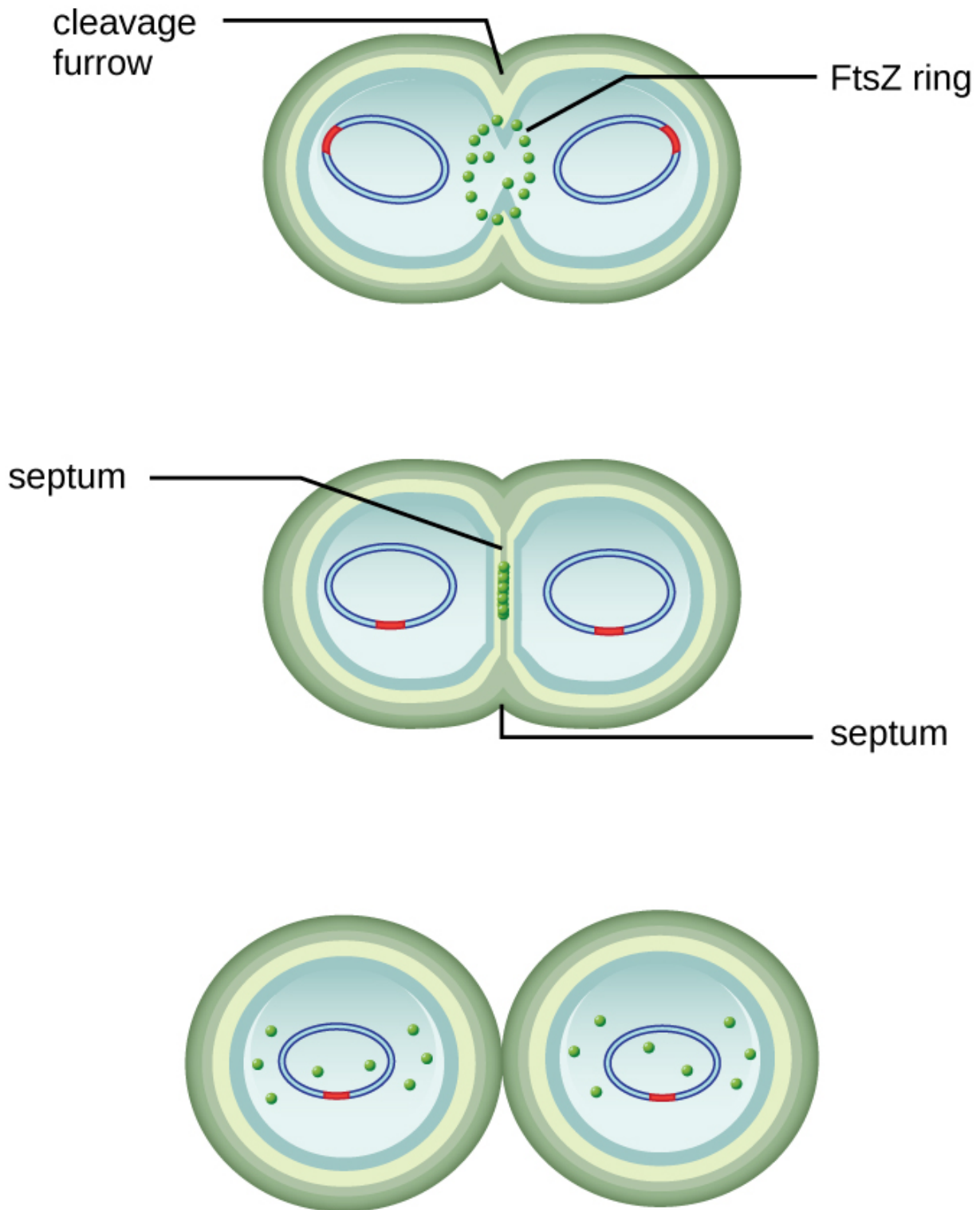


(a)



(b)

(a) The electron micrograph depicts two cells of *Salmonella typhimurium* after a binary fission event. (b) Binary fission in bacteria starts with the replication of DNA as the cell elongates. A division septum forms in the center of the cell. Two daughter cells of similar size form and separate, each receiving a copy of the original chromosome. (credit a: modification of work by Centers for Disease Control and Prevention)



FtsZ proteins assemble to form a Z ring that is anchored to the plasma membrane. The Z ring pinches the cell envelope to separate the cytoplasm of the new cells.

Note:

- What is the name of the protein that assembles into a Z ring to initiate cytokinesis and cell division?

Generation Time

In eukaryotic organisms, the generation time is the time between the same points of the life cycle in two successive generations. For example, the typical generation time for the human population is 25 years. This definition is not practical for bacteria, which may reproduce rapidly or remain dormant for thousands of years. In prokaryotes (Bacteria and Archaea), the **generation time** is also called the **doubling time** and is defined as the time it takes for the population to double through one round of binary fission. Bacterial doubling times vary enormously. Whereas *Escherichia coli* can double in as little as 20 minutes under optimal growth conditions in the laboratory, bacteria of the same species may need several days to double in especially harsh environments. Most pathogens grow rapidly, like *E. coli*, but there are exceptions. For example, *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has a generation time of between 15 and 20 hours. On the other hand, *M. leprae*, which causes Hansen's disease (leprosy), grows much more slowly, with a doubling time of 14 days.

Note:

Calculating Number of Cells

It is possible to predict the number of cells in a population when they divide by binary fission at a constant rate. As an example, consider what

happens if a single cell divides every 30 minutes for 24 hours. The diagram in [\[link\]](#) shows the increase in cell numbers for the first three generations. The number of cells increases exponentially and can be expressed as 2^n , where n is the number of generations. If cells divide every 30 minutes, after 24 hours, 48 divisions would have taken place. If we apply the formula 2^n , where n is equal to 48, the single cell would give rise to 2^{48} or 281,474,976,710,656 cells at 48 generations (24 hours). When dealing with such huge numbers, it is more practical to use scientific notation. Therefore, we express the number of cells as 2.8×10^{14} cells. In our example, we used one cell as the initial number of cells. For any number of starting cells, the formula is adapted as follows:

Equation:

$$N_n = N_0 2^n$$

N_n is the number of cells at any generation n , N_0 is the initial number of cells, and n is the number of generations.

Number of generations (n)	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

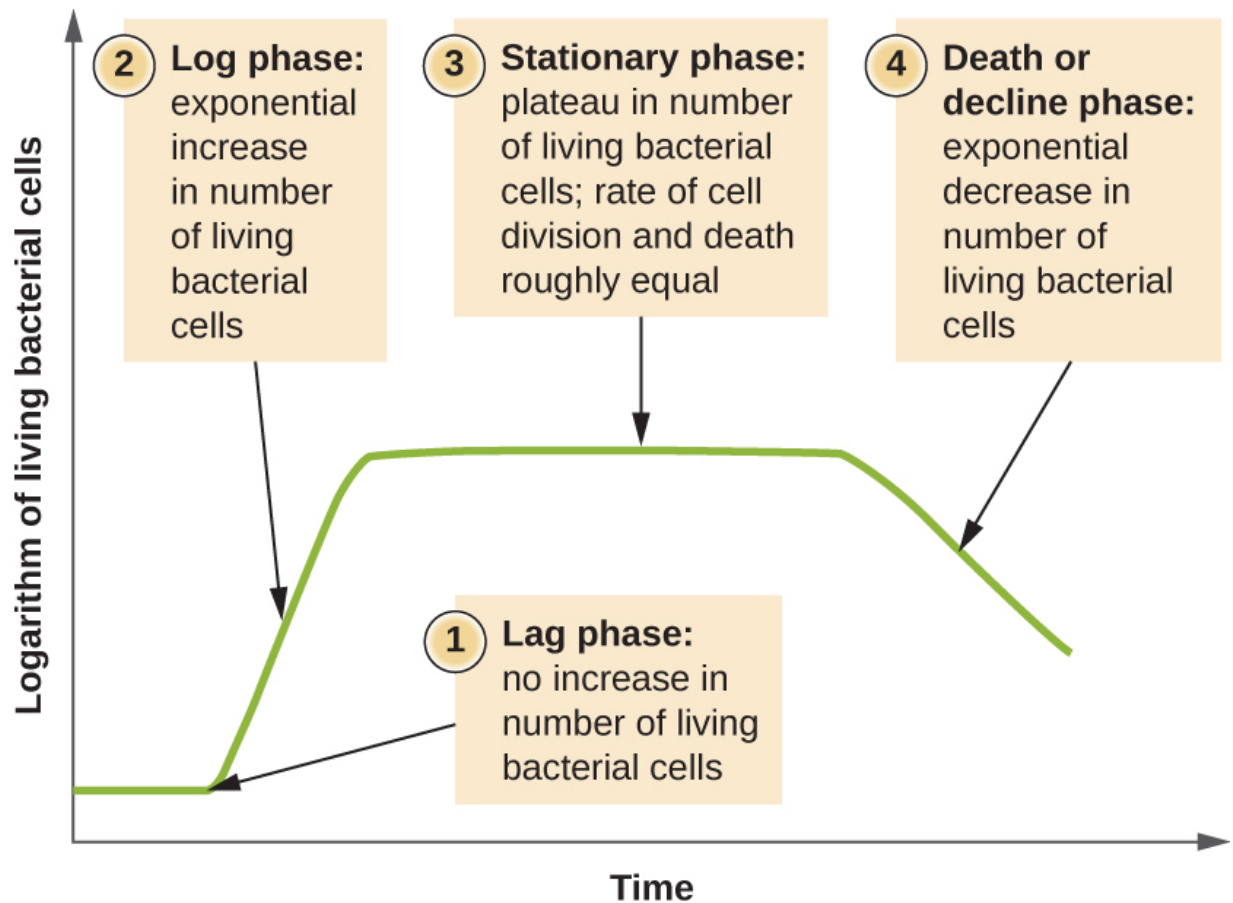
The parental cell divides and gives rise to two daughter cells. Each of the daughter cells, in turn, divides, giving a total of four cells in the second generation and eight cells in the third generation. Each division doubles the number of cells.

Note:

- With a doubling time of 30 minutes and a starting population size of 1×10^5 cells, how many cells will be present after 2 hours, assuming no cell death?

The Growth Curve

Microorganisms grown in closed culture (also known as a batch culture), in which no nutrients are added and most waste is not removed, follow a reproducible growth pattern referred to as the **growth curve**. An example of a batch culture in nature is a pond in which a small number of cells grow in a closed environment. The **culture density** is defined as the number of cells per unit volume. In a closed environment, the culture density is also a measure of the number of cells in the population. Infections of the body do not always follow the growth curve, but correlations can exist depending upon the site and type of infection. When the number of live cells is plotted against time, distinct phases can be observed in the curve ([link](#)).



The growth curve of a bacterial culture is represented by the logarithm of the number of live cells plotted as a function of time. The graph can be divided into four phases according to the slope, each of which matches events in the cell. The four phases are lag, log, stationary, and death.

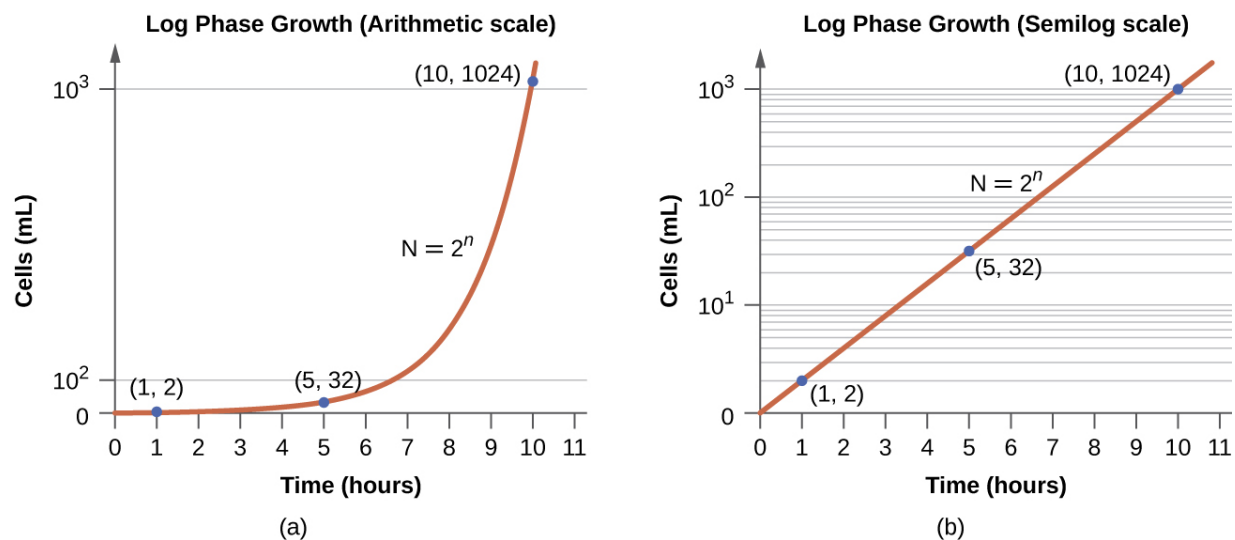
The Lag Phase

The beginning of the growth curve represents a small number of cells, referred to as an **inoculum**, that are added to a fresh **culture medium**, a nutritional broth that supports growth. The initial phase of the growth curve is called the **lag phase**, during which cells are gearing up for the next phase of growth. The number of cells does not change during the lag phase;

however, cells grow larger and are metabolically active, synthesizing proteins needed to grow within the medium. If any cells were damaged or shocked during the transfer to the new medium, repair takes place during the lag phase. The duration of the lag phase is determined by many factors, including the species and genetic make-up of the cells, the composition of the medium, and the size of the original inoculum.

The Log Phase

In the **logarithmic (log) growth phase**, sometimes called exponential growth phase, the cells are actively dividing by binary fission and their number increases exponentially. For any given bacterial species, the generation time under specific growth conditions (nutrients, temperature, pH, and so forth) is genetically determined, and this generation time is called the **intrinsic growth rate**. During the log phase, the relationship between time and number of cells is not linear but exponential; however, the growth curve is often plotted on a semilogarithmic graph, as shown in [\[link\]](#), which gives the appearance of a linear relationship.



Both graphs illustrate population growth during the log phase for a bacterial sample with an initial population of one cell and a doubling

time of 1 hour. (a) When plotted on an arithmetic scale, the growth rate resembles a curve. (b) When plotted on a semilogarithmic scale (meaning the values on the y-axis are logarithmic), the growth rate appears linear.

Cells in the log phase show constant growth rate and uniform metabolic activity. For this reason, cells in the log phase are preferentially used for industrial applications and research work. The log phase is also the stage where bacteria are the most susceptible to the action of disinfectants and common antibiotics that affect protein, DNA, and cell-wall synthesis.

Stationary Phase

As the number of cells increases through the log phase, several factors contribute to a slowing of the growth rate. Waste products accumulate and nutrients are gradually used up. In addition, gradual depletion of oxygen begins to limit aerobic cell growth. This combination of unfavorable conditions slows and finally stalls population growth. The total number of live cells reaches a plateau referred to as the **stationary phase** ([link](#)). In this phase, the number of new cells created by cell division is now equivalent to the number of cells dying; thus, the total population of living cells is relatively stagnant. The culture density in a stationary culture is constant. The culture's carrying capacity, or maximum culture density, depends on the types of microorganisms in the culture and the specific conditions of the culture; however, carrying capacity is constant for a given organism grown under the same conditions.

During the stationary phase, cells switch to a survival mode of metabolism. As growth slows, so too does the synthesis of peptidoglycans, proteins, and nucleic-acids; thus, stationary cultures are less susceptible to antibiotics that disrupt these processes. In bacteria capable of producing endospores, many cells undergo sporulation during the stationary phase. Secondary metabolites, including antibiotics, are synthesized in the stationary phase. In certain pathogenic bacteria, the stationary phase is also associated with the

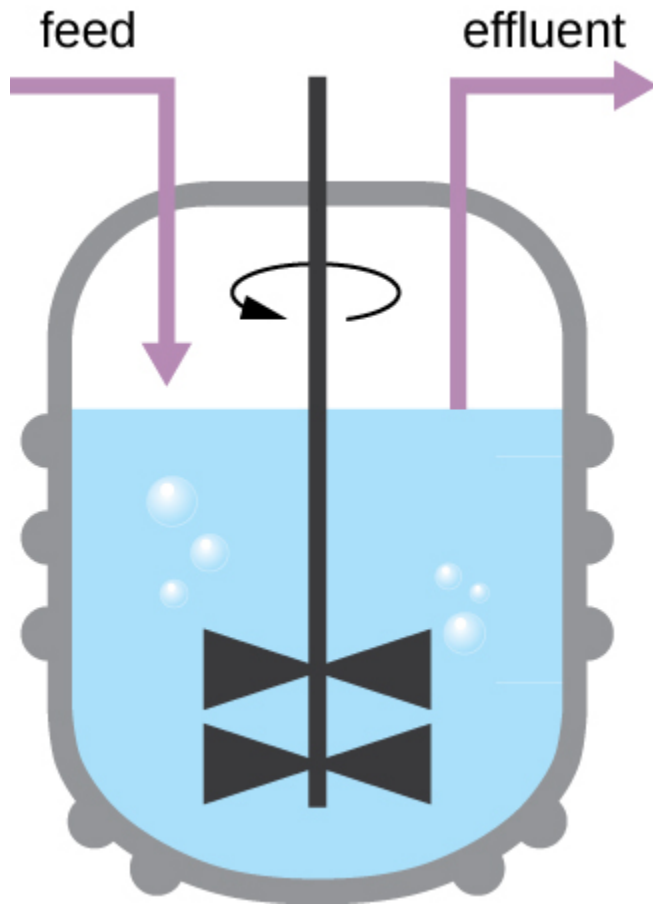
expression of virulence factors, products that contribute to a microbe's ability to survive, reproduce, and cause disease in a host organism. For example, quorum sensing in *Staphylococcus aureus* initiates the production of enzymes that can break down human tissue and cellular debris, clearing the way for bacteria to spread to new tissue where nutrients are more plentiful.

The Death Phase

As a culture medium accumulates toxic waste and nutrients are exhausted, cells die in greater and greater numbers. Soon, the number of dying cells exceeds the number of dividing cells, leading to an exponential decrease in the number of cells ([\[link\]](#)). This is the aptly named **death phase**, sometimes called the decline phase. Many cells lyse and release nutrients into the medium, allowing surviving cells to maintain viability and form endospores. A few cells, the so-called **persisters**, are characterized by a slow metabolic rate. Persister cells are medically important because they are associated with certain chronic infections, such as tuberculosis, that do not respond to antibiotic treatment.

Sustaining Microbial Growth

The growth pattern shown in [\[link\]](#) takes place in a closed environment; nutrients are not added and waste and dead cells are not removed. In many cases, though, it is advantageous to maintain cells in the logarithmic phase of growth. One example is in industries that harvest microbial products. A chemostat ([\[link\]](#)) is used to maintain a continuous culture in which nutrients are supplied at a steady rate. A controlled amount of air is mixed in for aerobic processes. Bacterial suspension is removed at the same rate as nutrients flow in to maintain an optimal growth environment.



A chemostat is a culture vessel fitted with an opening to add nutrients (feed) and an outlet to remove contents (effluent), effectively diluting toxic wastes and dead cells. The addition and removal of fluids is adjusted to maintain the culture in the logarithmic phase of growth. If aerobic bacteria are grown, suitable oxygen levels are maintained.

Note:

- During which phase does growth occur at the fastest rate?
- Name two factors that limit microbial growth.

Measurement of Bacterial Growth

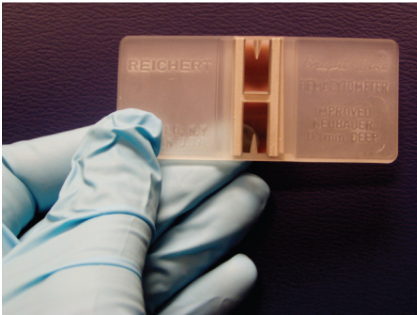
Estimating the number of bacterial cells in a sample, known as a bacterial count, is a common task performed by microbiologists. The number of bacteria in a clinical sample serves as an indication of the extent of an infection. Quality control of drinking water, food, medication, and even cosmetics relies on estimates of bacterial counts to detect contamination and prevent the spread of disease. Two major approaches are used to measure cell number. The direct methods involve counting cells, whereas the indirect methods depend on the measurement of cell presence or activity without actually counting individual cells. Both direct and indirect methods have advantages and disadvantages for specific applications.

Direct Cell Count

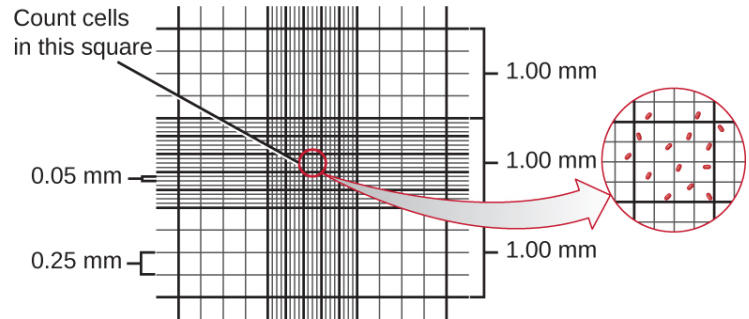
Direct cell count refers to counting the cells in a liquid culture or colonies on a plate. It is a direct way of estimating how many organisms are present in a sample. Let's look first at a simple and fast method that requires only a specialized slide and a compound microscope.

The simplest way to count bacteria is called the **direct microscopic cell count**, which involves transferring a known volume of a culture to a calibrated slide and counting the cells under a light microscope. The calibrated slide is called a **Petroff-Hausser chamber** ([\[link\]](#)) and is similar to a hemocytometer used to count red blood cells. The central area of the counting chamber is etched into squares of various sizes. A sample of the culture suspension is added to the chamber under a coverslip that is placed at a specific height from the surface of the grid. It is possible to estimate the

concentration of cells in the original sample by counting individual cells in a number of squares and determining the volume of the sample observed. The area of the squares and the height at which the coverslip is positioned are specified for the chamber. The concentration must be corrected for dilution if the sample was diluted before enumeration.



(a)

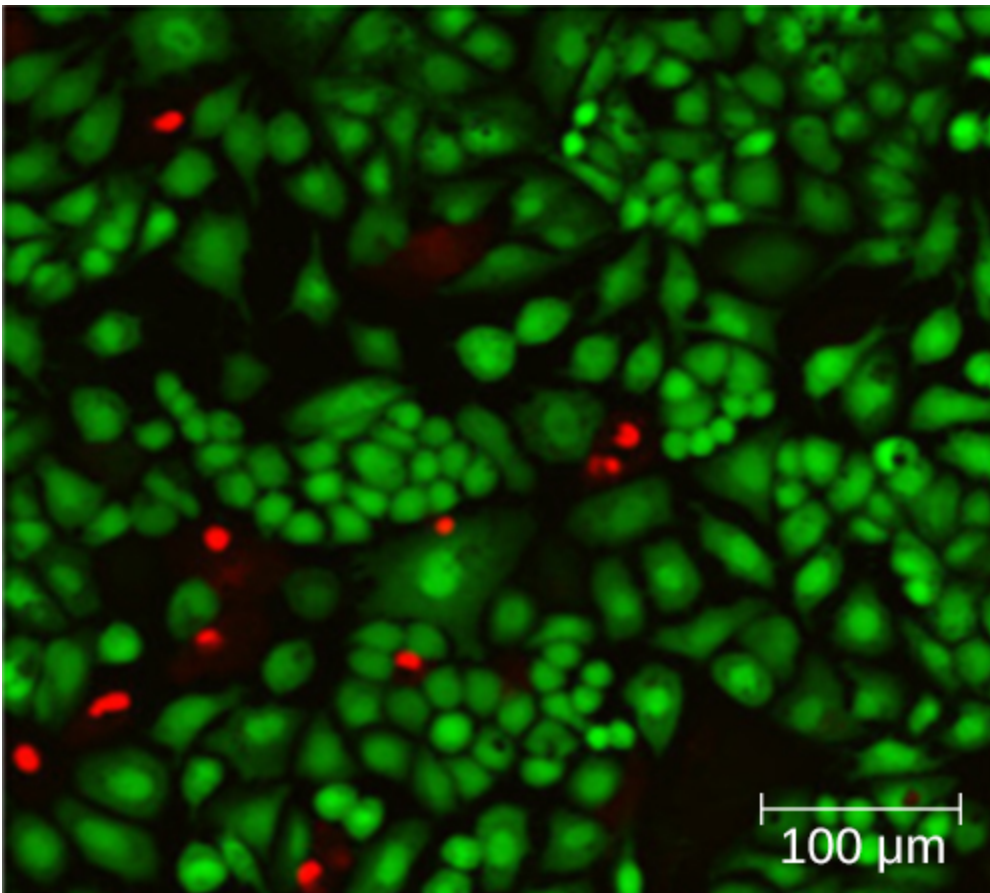


(b)

(a) A Petroff-Hausser chamber is a special slide designed for counting the bacterial cells in a measured volume of a sample. A grid is etched on the slide to facilitate precision in counting. (b) This diagram illustrates the grid of a Petroff-Hausser chamber, which is made up of squares of known areas. The enlarged view shows the square within which bacteria (red cells) are counted. If the coverslip is 0.2 mm above the grid and the square has an area of 0.04 mm^2 , then the volume is 0.008 mm^3 , or 0.000008 mL . Since there are 10 cells inside the square, the density of bacteria is $10 \text{ cells}/0.000008 \text{ mL}$, which equates to $1,250,000 \text{ cells/mL}$. (credit a: modification of work by Jeffrey M. Vinocur)

Cells in several small squares must be counted and the average taken to obtain a reliable measurement. The advantages of the chamber are that the method is easy to use, relatively fast, and inexpensive. On the downside, the counting chamber does not work well with dilute cultures because there may not be enough cells to count.

Using a counting chamber does not necessarily yield an accurate count of the number of live cells because it is not always possible to distinguish between live cells, dead cells, and debris of the same size under the microscope. However, newly developed fluorescence staining techniques make it possible to distinguish viable and dead bacteria. These viability stains (or live stains) bind to nucleic acids, but the primary and secondary stains differ in their ability to cross the cytoplasmic membrane. The primary stain, which fluoresces green, can penetrate intact cytoplasmic membranes, staining both live and dead cells. The secondary stain, which fluoresces red, can stain a cell only if the cytoplasmic membrane is considerably damaged. Thus, live cells fluoresce green because they only absorb the green stain, whereas dead cells appear red because the red stain displaces the green stain on their nucleic acids ([link](#)).

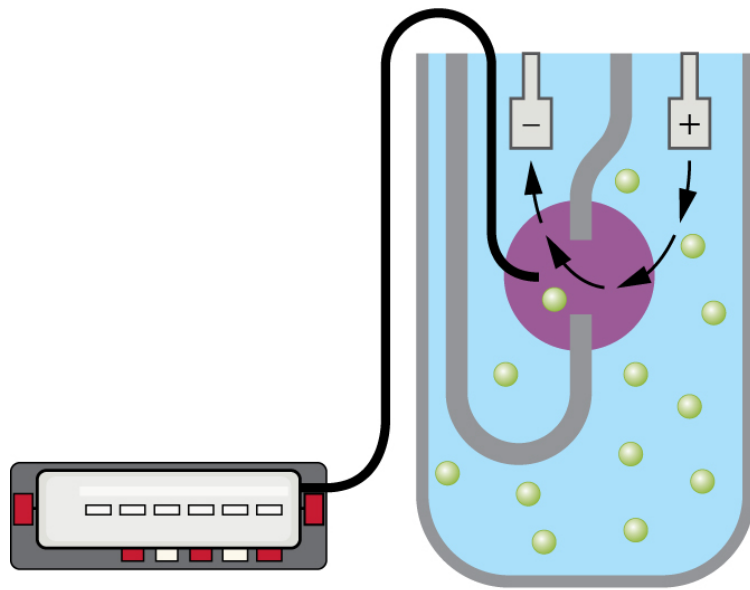


Fluorescence staining can be used to differentiate

between viable and dead bacterial cells in a sample for purposes of counting. Viable cells are stained green, whereas dead cells are stained red. (credit: modification of work by Panseri S, Cunha C, D'Alessandro T, Sandri M, Giavaresi G, Maracci M, Hung CT, Tampieri A)

Another technique uses an electronic cell counting device (Coulter counter) to detect and count the changes in electrical resistance in a saline solution. A glass tube with a small opening is immersed in an electrolyte solution. A first electrode is suspended in the glass tube. A second electrode is located outside of the tube. As cells are drawn through the small aperture in the glass tube, they briefly change the resistance measured between the two electrodes and the change is recorded by an electronic sensor ([\[link\]](#)); each resistance change represents a cell. The method is rapid and accurate within a range of concentrations; however, if the culture is too concentrated, more than one cell may pass through the aperture at any given time and skew the results. This method also does not differentiate between live and dead cells.

Direct counts provide an estimate of the total number of cells in a sample. However, in many situations, it is important to know the number of live, or **viable**, cells. Counts of live cells are needed when assessing the extent of an infection, the effectiveness of antimicrobial compounds and medication, or contamination of food and water.



(a)



(b)

A Coulter counter is an electronic device that counts cells. It measures the change in resistance in an electrolyte solution that takes place when a cell passes through a small opening in the inside container wall. A detector automatically counts the number of cells passing through the opening. (credit b: modification of work by National Institutes of Health)

Note:

- Why would you count the number of cells in more than one square in the Petroff-Hausser chamber to estimate cell numbers?
- In the viability staining method, why do dead cells appear red?

Plate Count

The **viable plate count**, or simply plate count, is a count of viable or live cells. It is based on the principle that viable cells replicate and give rise to visible colonies when incubated under suitable conditions for the specimen. The results are usually expressed as **colony-forming units** per milliliter (CFU/mL) rather than cells per milliliter because more than one cell may have landed on the same spot to give rise to a single colony. Furthermore, samples of bacteria that grow in clusters or chains are difficult to disperse and a single colony may represent several cells. Some cells are described as viable but nonculturable and will not form colonies on solid media. For all these reasons, the viable plate count is considered a low estimate of the actual number of live cells. These limitations do not detract from the usefulness of the method, which provides estimates of live bacterial numbers.

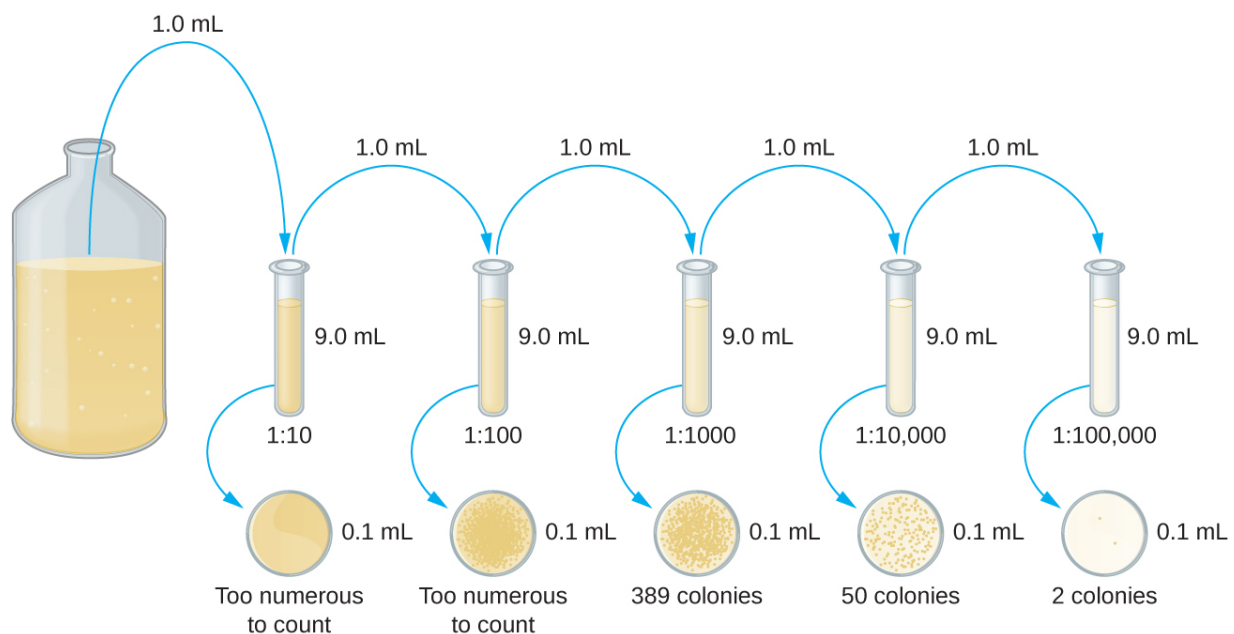
Microbiologists typically count plates with 30–300 colonies. Samples with too few colonies (<30) do not give statistically reliable numbers, and overcrowded plates (>300 colonies) make it difficult to accurately count individual colonies. Also, counts in this range minimize occurrences of more than one bacterial cell forming a single colony. Thus, the calculated CFU is closer to the true number of live bacteria in the population.

There are two common approaches to inoculating plates for viable counts: the pour plate and the spread plate methods. Although the final inoculation procedure differs between these two methods, they both start with a serial dilution of the culture.

Serial Dilution

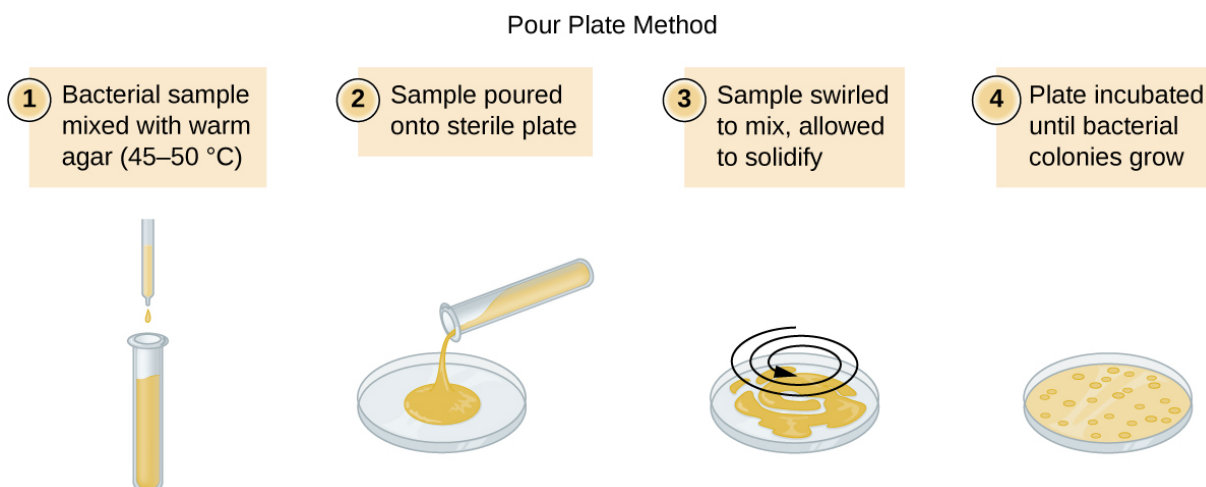
The **serial dilution** of a culture is an important first step before proceeding to either the pour plate or spread plate method. The goal of the serial dilution process is to obtain plates with CFUs in the range of 30–300, and the process usually involves several dilutions in multiples of 10 to simplify calculation. The number of serial dilutions is chosen according to a preliminary estimate of the culture density. [\[link\]](#) illustrates the serial dilution method.

A fixed volume of the original culture, 1.0 mL, is added to and thoroughly mixed with the first dilution tube solution, which contains 9.0 mL of sterile broth. This step represents a dilution factor of 10, or 1:10, compared with the original culture. From this first dilution, the same volume, 1.0 mL, is withdrawn and mixed with a fresh tube of 9.0 mL of dilution solution. The dilution factor is now 1:100 compared with the original culture. This process continues until a series of dilutions is produced that will bracket the desired cell concentration for accurate counting. From each tube, a sample is plated on solid medium using either the **pour plate method** ([\[link\]](#)) or the **spread plate method** ([\[link\]](#)). The plates are incubated until colonies appear. Two to three plates are usually prepared from each dilution and the numbers of colonies counted on each plate are averaged. In all cases, thorough mixing of samples with the dilution medium (to ensure the cell distribution in the tube is random) is paramount to obtaining reliable results.

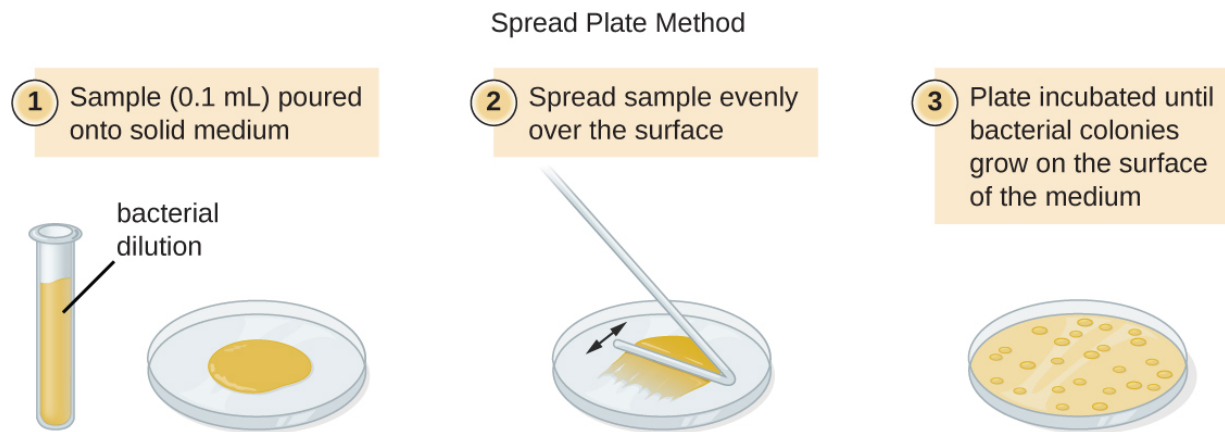


Serial dilution involves diluting a fixed volume of cells mixed with dilution solution using the previous dilution as an inoculum. The result is dilution of the original culture by an exponentially growing factor. (credit: modification of work by “Leberecht”/Wikimedia Commons)

The dilution factor is used to calculate the number of cells in the original cell culture. In our example, an average of 50 colonies was counted on the plates obtained from the 1:10,000 dilution. Because only 0.1 mL of suspension was pipetted on the plate, the multiplier required to reconstitute the original concentration is $10 \times 10,000$. The number of CFU per mL is equal to $50 \times 10 \times 10,000 = 5,000,000$. The number of bacteria in the culture is estimated as 5 million cells/mL. The colony count obtained from the 1:1000 dilution was 389, well below the expected 500 for a 10-fold difference in dilutions. This highlights the issue of inaccuracy when colony counts are greater than 300 and more than one bacterial cell grows into a single colony.



In the pour plate method of cell counting, the sample is mixed in liquid warm agar (45–50 °C) poured into a sterile Petri dish and further mixed by swirling. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number of cells in the original volume sampled.



In the spread plate method of cell counting, the sample is poured onto solid agar and then spread using a sterile spreader. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number of cells in the original volume samples.

A very dilute sample—drinking water, for example—may not contain enough organisms to use either of the plate count methods described. In such cases, the original sample must be concentrated rather than diluted before plating. This can be accomplished using a modification of the plate count technique called the **membrane filtration technique**. Known volumes are vacuum-filtered aseptically through a membrane with a pore size small enough to trap microorganisms. The membrane is transferred to a Petri plate containing an appropriate growth medium. Colonies are counted after incubation. Calculation of the cell density is made by dividing the cell count by the volume of filtered liquid.

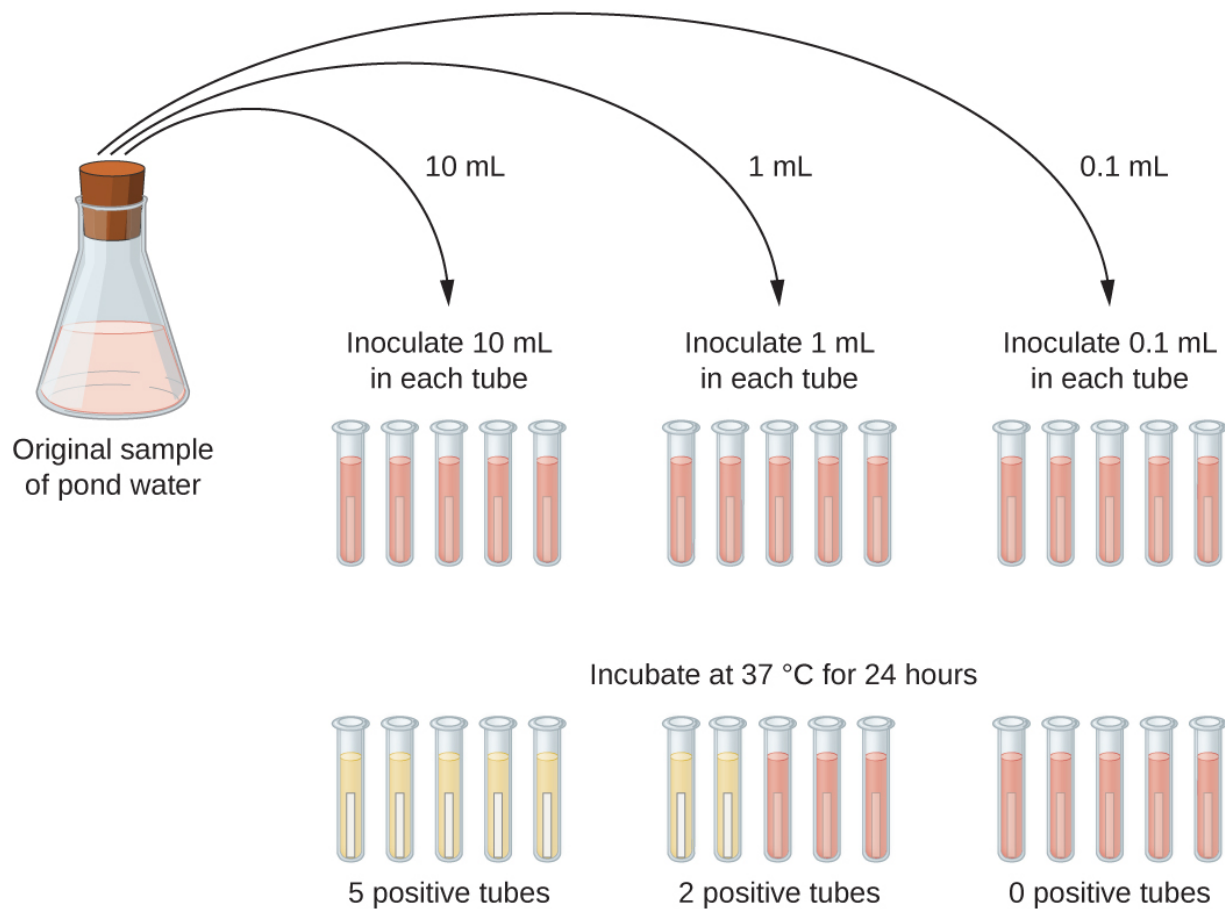
Note:

Watch this [video](#) for demonstrations of serial dilutions and spread plate techniques.

The Most Probable Number

The number of microorganisms in dilute samples is usually too low to be detected by the plate count methods described thus far. For these specimens, microbiologists routinely use the **most probable number (MPN) method**, a statistical procedure for estimating of the number of viable microorganisms in a sample. Often used for water and food samples, the MPN method evaluates detectable growth by observing changes in turbidity or color due to metabolic activity.

A typical application of MPN method is the estimation of the number of coliforms in a sample of pond water. Coliforms are gram-negative rod bacteria that ferment lactose. The presence of coliforms in water is considered a sign of contamination by fecal matter. For the method illustrated in [\[link\]](#), a series of three dilutions of the water sample is tested by inoculating five lactose broth tubes with 10 mL of sample, five lactose broth tubes with 1 mL of sample, and five lactose broth tubes with 0.1 mL of sample. The lactose broth tubes contain a pH indicator that changes color from red to yellow when the lactose is fermented. After inoculation and incubation, the tubes are examined for an indication of coliform growth by a color change in media from red to yellow. The first set of tubes (10-mL sample) showed growth in all the tubes; the second set of tubes (1 mL) showed growth in two tubes out of five; in the third set of tubes, no growth is observed in any of the tubes (0.1-mL dilution). The numbers 5, 2, and 0 are compared with [Figure B1](#) in [Appendix B](#), which has been constructed using a probability model of the sampling procedure. From our reading of the table, we conclude that 49 is the most probable number of bacteria per 100 mL of pond water.



In the most probable number method, sets of five lactose broth tubes are inoculated with three different volumes of pond water: 10 mL, 1 mL, and 0.1mL. Bacterial growth is assessed through a change in the color of the broth from red to yellow as lactose is fermented.

Note:

- What is a colony-forming unit?
- What two methods are frequently used to estimate bacterial numbers in water samples?

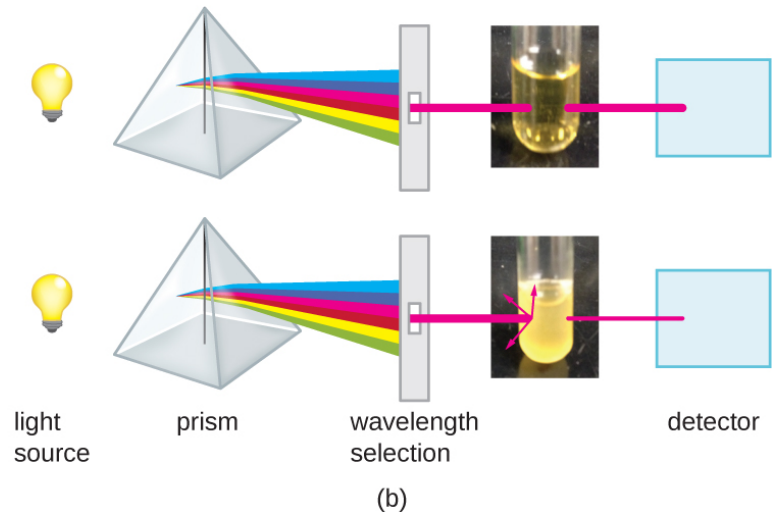
Indirect Cell Counts

Besides direct methods of counting cells, other methods, based on an indirect detection of cell density, are commonly used to estimate and compare cell densities in a culture. The foremost approach is to measure the **turbidity** (cloudiness) of a sample of bacteria in a liquid suspension. The laboratory instrument used to measure turbidity is called a spectrophotometer ([link](#)). In a spectrophotometer, a light beam is transmitted through a bacterial suspension, the light passing through the suspension is measured by a detector, and the amount of light passing through the sample and reaching the detector is converted to either percent transmission or a logarithmic value called absorbance (optical density). As the numbers of bacteria in a suspension increase, the turbidity also increases and causes less light to reach the detector. The decrease in light passing through the sample and reaching the detector is associated with a decrease in percent transmission and increase in absorbance measured by the spectrophotometer.

Measuring turbidity is a fast method to estimate cell density as long as there are enough cells in a sample to produce turbidity. It is possible to correlate turbidity readings to the actual number of cells by performing a viable plate count of samples taken from cultures having a range of absorbance values. Using these values, a calibration curve is generated by plotting turbidity as a function of cell density. Once the calibration curve has been produced, it can be used to estimate cell counts for all samples obtained or cultured under similar conditions and with densities within the range of values used to construct the curve.



(a)



(a) A spectrophotometer is commonly used to measure the turbidity of a bacterial cell suspension as an indirect measure of cell density. (b) A spectrophotometer works by splitting white light from a source into a spectrum. The spectrophotometer allows choice of the wavelength of light to use for the measurement. The optical density (turbidity) of the sample will depend on the wavelength, so once one wavelength is chosen, it must be used consistently. The filtered light passes through the sample (or a control with only medium) and the light intensity is measured by a detector. The light passing into a suspension of bacteria is scattered by the cells in such a way that some fraction of it never reaches the detector. This scattering happens to a far lesser degree in the control tube with only the medium. (credit a: modification of work by Hwang HS, Kim MS; credit b “test tube photos”: modification of work by Suzanne Wakim)

Measuring dry weight of a culture sample is another indirect method of evaluating culture density without directly measuring cell counts. The cell suspension used for weighing must be concentrated by filtration or centrifugation, washed, and then dried before the measurements are taken. The degree of drying must be standardized to account for residual water content. This method is especially useful for filamentous microorganisms, which are difficult to enumerate by direct or viable plate count.

As we have seen, methods to estimate viable cell numbers can be labor intensive and take time because cells must be grown. Recently, indirect ways of measuring live cells have been developed that are both fast and easy to implement. These methods measure cell activity by following the production of metabolic products or disappearance of reactants. Adenosine triphosphate (ATP) formation, biosynthesis of proteins and nucleic acids, and consumption of oxygen can all be monitored to estimate the number of cells.

Note:

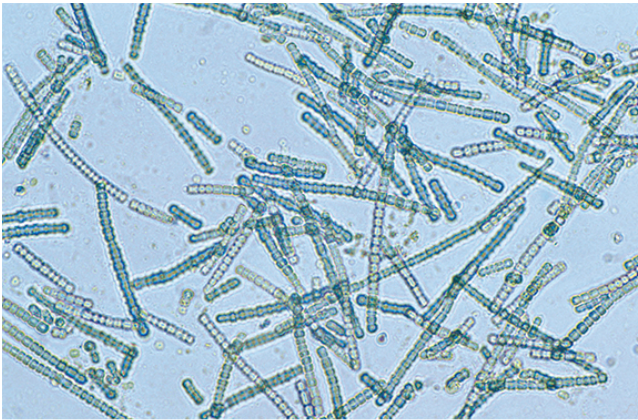
- What is the purpose of a calibration curve when estimating cell count from turbidity measurements?
- What are the newer indirect methods of counting live cells?

Alternative Patterns of Cell Division

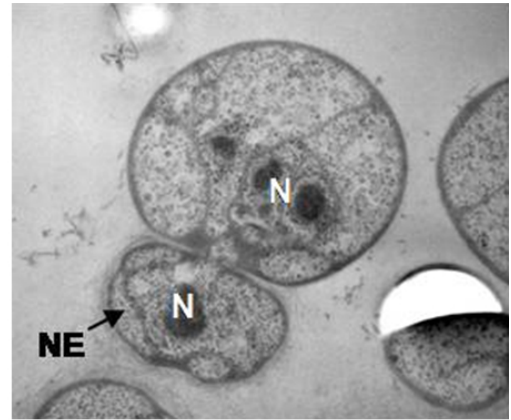
Binary fission is the most common pattern of cell division in prokaryotes, but it is not the only one. Other mechanisms usually involve asymmetrical division (as in budding) or production of spores in aerial filaments.

In some cyanobacteria, many nucleoids may accumulate in an enlarged round cell or along a filament, leading to the generation of many new cells at once. The new cells often split from the parent filament and float away in a process called **fragmentation** ([\[link\]](#)). Fragmentation is commonly observed in the Actinomycetes, a group of gram-positive, anaerobic bacteria commonly found in soil. Another curious example of cell division in prokaryotes, reminiscent of live birth in animals, is exhibited by the giant bacterium *Epulopiscium*. Several daughter cells grow fully in the parent cell, which eventually disintegrates, releasing the new cells to the environment. Other species may form a long narrow extension at one pole in a process called **budding**. The tip of the extension swells and forms a smaller cell, the bud that eventually detaches from the parent cell. Budding

is most common in yeast ([link](#)), but it is also observed in prosthecate bacteria and some cyanobacteria.



(a)



(b)

(a) Filamentous cyanobacteria, like those pictured here, replicate by fragmentation. (b) In this electron micrograph, cells of the bacterium *Gemmata obscuriglobus* are budding. The larger cell is the mother cell. Labels indicate the nucleoids (N) and the still-forming nuclear envelope (NE) of the daughter cell. (credit a: modification of work by CSIRO; credit b: modification of work by Kuo-Chang Lee, Rick I Webb and John A Fuerst)

The soil bacteria *Actinomyces* grow in long filaments divided by septa, similar to the mycelia seen in fungi, resulting in long cells with multiple nucleoids. Environmental signals, probably related to low nutrient availability, lead to the formation of aerial filaments. Within these aerial filaments, elongated cells divide simultaneously. The new cells, which contain a single nucleoid, develop into spores that give rise to new colonies.

Note:

- Identify at least one difference between fragmentation and budding.

Biofilms

In nature, microorganisms grow mainly in **biofilms**, complex and dynamic ecosystems that form on a variety of environmental surfaces, from industrial conduits and water treatment pipelines to rocks in river beds. Biofilms are not restricted to solid surface substrates, however. Almost any surface in a liquid environment containing some minimal nutrients will eventually develop a biofilm. Microbial mats that float on water, for example, are biofilms that contain large populations of photosynthetic microorganisms. Biofilms found in the human mouth may contain hundreds of bacterial species. Regardless of the environment where they occur, biofilms are not random collections of microorganisms; rather, they are highly structured communities that provide a selective advantage to their constituent microorganisms.

Biofilm Structure

Observations using confocal microscopy have shown that environmental conditions influence the overall structure of biofilms. Filamentous biofilms called streamers form in rapidly flowing water, such as freshwater streams, eddies, and specially designed laboratory flow cells that replicate growth conditions in fast-moving fluids. The streamers are anchored to the substrate by a “head” and the “tail” floats downstream in the current. In still or slow-moving water, biofilms mainly assume a mushroom-like shape. The structure of biofilms may also change with other environmental conditions such as nutrient availability.

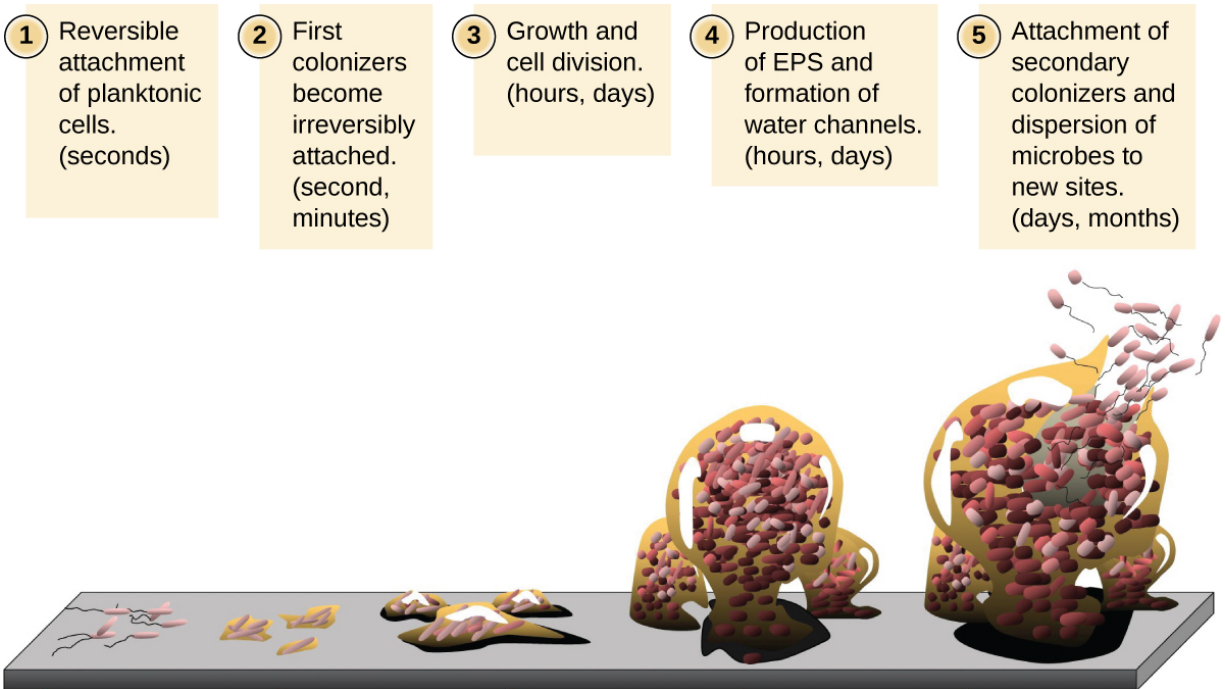
Detailed observations of biofilms under confocal laser and scanning electron microscopes reveal clusters of microorganisms embedded in a matrix interspersed with open water channels. The extracellular matrix consists of **extracellular polymeric substances (EPS)** secreted by the

organisms in the biofilm. The extracellular matrix represents a large fraction of the biofilm, accounting for 50%–90% of the total dry mass. The properties of the EPS vary according to the resident organisms and environmental conditions.

EPS is a hydrated gel composed primarily of polysaccharides and containing other macromolecules such as proteins, nucleic acids, and lipids. It plays a key role in maintaining the integrity and function of the biofilm. Channels in the EPS allow movement of nutrients, waste, and gases throughout the biofilm. This keeps the cells hydrated, preventing desiccation. EPS also shelters organisms in the biofilm from predation by other microbes or cells (e.g., protozoans, white blood cells in the human body).

Biofilm Formation

Free-floating microbial cells that live in an aquatic environment are called **planktonic** cells. The formation of a biofilm essentially involves the attachment of planktonic cells to a substrate, where they become **sessile** (attached to a surface). This occurs in stages, as depicted in [\[link\]](#). The first stage involves the attachment of planktonic cells to a surface coated with a conditioning film of organic material. At this point, attachment to the substrate is reversible, but as cells express new phenotypes that facilitate the formation of EPS, they transition from a planktonic to a sessile lifestyle. The biofilm develops characteristic structures, including an extensive matrix and water channels. Appendages such as fimbriae, pili, and flagella interact with the EPS, and microscopy and genetic analysis suggest that such structures are required for the establishment of a mature biofilm. In the last stage of the biofilm life cycle, cells on the periphery of the biofilm revert to a planktonic lifestyle, sloughing off the mature biofilm to colonize new sites. This stage is referred to as dispersal.



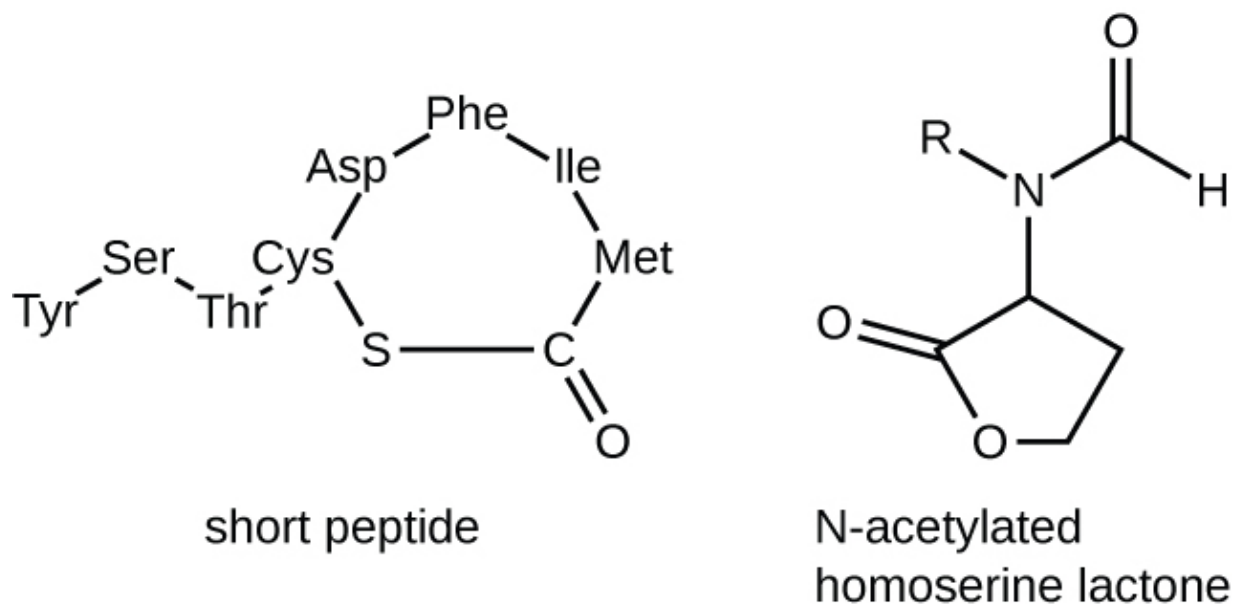
Stages in the formation and life cycle of a biofilm. (credit: modification of work by Public Library of Science and American Society for Microbiology)

Within a biofilm, different species of microorganisms establish metabolic collaborations in which the waste product of one organism becomes the nutrient for another. For example, aerobic microorganisms consume oxygen, creating anaerobic regions that promote the growth of anaerobes. This occurs in many polymicrobial infections that involve both aerobic and anaerobic pathogens.

The mechanism by which cells in a biofilm coordinate their activities in response to environmental stimuli is called **quorum sensing**. Quorum sensing—which can occur between cells of different species within a biofilm—enables microorganisms to detect their cell density through the release and binding of small, diffusible molecules called **autoinducers**. When the cell population reaches a critical threshold (a quorum), these autoinducers initiate a cascade of reactions that activate genes associated with cellular functions that are beneficial only when the population reaches

a critical density. For example, in some pathogens, synthesis of virulence factors only begins when enough cells are present to overwhelm the immune defenses of the host. Although mostly studied in bacterial populations, quorum sensing takes place between bacteria and eukaryotes and between eukaryotic cells such as the fungus *Candida albicans*, a common member of the human microbiota that can cause infections in immunocompromised individuals.

The signaling molecules in quorum sensing belong to two major classes. Gram-negative bacteria communicate mainly using N-acylated homoserine lactones, whereas gram-positive bacteria mostly use small peptides ([\[link\]](#)). In all cases, the first step in quorum sensing consists of the binding of the autoinducer to its specific receptor only when a threshold concentration of signaling molecules is reached. Once binding to the receptor takes place, a cascade of signaling events leads to changes in gene expression. The result is the activation of biological responses linked to quorum sensing, notably an increase in the production of signaling molecules themselves, hence the term autoinducer.



Short peptides in gram-positive bacteria and N-acylated homoserine lactones in gram-negative bacteria act as autoinducers in quorum

sensing and mediate the coordinated response of bacterial cells. The R side chain of the N-acetylated homoserine lactone is specific for the species of gram-negative bacteria. Some secreted homoserine lactones are recognized by more than one species.

Biofilms and Human Health

The human body harbors many types of biofilms, some beneficial and some harmful. For example, the layers of normal microbiota lining the intestinal and respiratory mucosa play a role in warding off infections by pathogens. However, other biofilms in the body can have a detrimental effect on health. For example, the plaque that forms on teeth is a biofilm that can contribute to dental and periodontal disease. Biofilms can also form in wounds, sometimes causing serious infections that can spread. The bacterium *Pseudomonas aeruginosa* often colonizes biofilms in the airways of patients with cystic fibrosis, causing chronic and sometimes fatal infections of the lungs. Biofilms can also form on medical devices used in or on the body, causing infections in patients with in-dwelling catheters, artificial joints, or contact lenses.

Pathogens embedded within biofilms exhibit a higher resistance to antibiotics than their free-floating counterparts. Several hypotheses have been proposed to explain why. Cells in the deep layers of a biofilm are metabolically inactive and may be less susceptible to the action of antibiotics that disrupt metabolic activities. The EPS may also slow the diffusion of antibiotics and antiseptics, preventing them from reaching cells in the deeper layers of the biofilm. Phenotypic changes may also contribute to the increased resistance exhibited by bacterial cells in biofilms. For example, the increased production of efflux pumps, membrane-embedded proteins that actively extrude antibiotics out of bacterial cells, have been shown to be an important mechanism of antibiotic resistance among biofilm-associated bacteria. Finally, biofilms provide an ideal environment for the exchange of extrachromosomal DNA, which often includes genes that confer antibiotic resistance.

Note:

- What is the matrix of a biofilm composed of?
- What is the role of quorum sensing in a biofilm?

Key Concepts and Summary

- Most bacterial cells divide by **binary fission**. **Generation time** in bacterial growth is defined as the **doubling time** of the population.
- Cells in a closed system follow a pattern of growth with four phases: **lag**, **logarithmic (exponential)**, **stationary**, and **death**.
- Cells can be counted by **direct viable cell count**. The **pour plate** and **spread plate** methods are used to plate **serial dilutions** into or onto, respectively, agar to allow counting of viable cells that give rise to **colony-forming units**. **Membrane filtration** is used to count live cells in dilute solutions. The **most probable cell number (MPN)** method allows estimation of cell numbers in cultures without using solid media.
- Indirect methods can be used to estimate **culture density** by measuring **turbidity** of a culture or live cell density by measuring metabolic activity.
- Other patterns of cell division include multiple nucleoid formation in cells; asymmetric division, as in **budding**; and the formation of hyphae and terminal spores.
- **Biofilms** are communities of microorganisms enmeshed in a matrix of **extracellular polymeric substance**. The formation of a biofilm occurs when **planktonic** cells attach to a substrate and become **sessile**. Cells in biofilms coordinate their activity by communicating through **quorum sensing**.
- Biofilms are commonly found on surfaces in nature and in the human body, where they may be beneficial or cause severe infections. Pathogens associated with biofilms are often more resistant to antibiotics and disinfectants.

Multiple Choice

Exercise:

Problem:

Which of the following methods would be used to measure the concentration of bacterial contamination in processed peanut butter?

- A. turbidity measurement
- B. total plate count
- C. dry weight measurement
- D. direct counting of bacteria on a calibrated slide under the microscope

Solution:

B

Exercise:

Problem:

In which phase would you expect to observe the most endospores in a *Bacillus* cell culture?

- A. death phase
- B. lag phase
- C. log phase
- D. log, lag, and death phases would all have roughly the same number of endospores.

Solution:

A

Exercise:

Problem:

During which phase would penicillin, an antibiotic that inhibits cell-wall synthesis, be most effective?

- A. death phase
- B. lag phase
- C. log phase
- D. stationary phase

Solution:

C

Exercise:**Problem:**

Which of the following is the best definition of generation time in a bacterium?

- A. the length of time it takes to reach the log phase
- B. the length of time it takes for a population of cells to double
- C. the time it takes to reach stationary phase
- D. the length of time of the exponential phase

Solution:

B

Exercise:

Problem: What is the function of the Z ring in binary fission?

- A. It controls the replication of DNA.
- B. It forms a contractile ring at the septum.
- C. It separates the newly synthesized DNA molecules.

D. It mediates the addition of new peptidoglycan subunits.

Solution:

B

Exercise:

Problem:

If a culture starts with 50 cells, how many cells will be present after five generations with no cell death?

- A. 200
 - B. 400
 - C. 1600
 - D. 3200
-

Solution:

C

Exercise:

Problem:

Filamentous cyanobacteria often divide by which of the following?

- A. budding
 - B. mitosis
 - C. fragmentation
 - D. formation of endospores
-

Solution:

C

Exercise:

Problem:

Which is a reason for antimicrobial resistance being higher in a biofilm than in free-floating bacterial cells?

- A. The EPS allows faster diffusion of chemicals in the biofilm.
- B. Cells are more metabolically active at the base of a biofilm.
- C. Cells are metabolically inactive at the base of a biofilm.
- D. The structure of a biofilm favors the survival of antibiotic resistant cells.

Solution:

C

Exercise:**Problem:**

Quorum sensing is used by bacterial cells to determine which of the following?

- A. the size of the population
- B. the availability of nutrients
- C. the speed of water flow
- D. the density of the population

Solution:

D

Exercise:**Problem:**

Which of the following statements about autoinducers is incorrect?

- A. They bind directly to DNA to activate transcription.

- B. They can activate the cell that secreted them.
 - C. N-acylated homoserine lactones are autoinducers in gram-negative cells.
 - D. Autoinducers may stimulate the production of virulence factors.
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

Direct count of total cells can be performed using a _____ or a _____.

Solution:

hemocytometer, Petroff-Hausser counting chamber

Exercise:

Problem:

The _____ method allows direct count of total cells growing on solid medium.

Solution:

plate count

Exercise:

Problem:

A statistical estimate of the number of live cells in a liquid is usually done by _____.

Solution:

most probable number

Exercise:**Problem:**

For this indirect method of estimating the growth of a culture, you measure _____ using a spectrophotometer.

Solution:

turbidity

Exercise:**Problem:**

Active growth of a culture may be estimated indirectly by measuring the following products of cell metabolism: _____ or _____.

Solution:

ATP, acid from fermentation

Matching**Exercise:****Problem:**

Match the definition with the name of the growth phase in the growth curve.

--	--

___Number of dying cells is higher than the number of cells dividing	A. Lag phase
___Number of new cells equal to number of dying cells	B. Log phase
___New enzymes to use available nutrients are induced	C. Stationary phase
___Binary fission is occurring at maximum rate	D. Death phase

Solution:

D, C, A, B

Short Answer

Exercise:

Problem:

Why is it important to measure the transmission of light through a control tube with only broth in it when making turbidity measures of bacterial cultures?

Exercise:

Problem:

In terms of counting cells, what does a plating method accomplish that an electronic cell counting method does not?

Exercise:

Problem:

Order the following stages of the development of a biofilm from the earliest to the last step.

1. secretion of EPS
2. reversible attachment
3. dispersal
4. formation of water channels
5. irreversible attachment

Exercise:**Problem:**

Infections among hospitalized patients are often related to the presence of a medical device in the patient. Which conditions favor the formation of biofilms on in-dwelling catheters and prostheses?

Critical Thinking**Exercise:**

Problem:

A patient in the hospital has an intravenous catheter inserted to allow for the delivery of medications, fluids, and electrolytes. Four days after the catheter is inserted, the patient develops a fever and an infection in the skin around the catheter. Blood cultures reveal that the patient has a blood-borne infection. Tests in the clinical laboratory identify the blood-borne pathogen as *Staphylococcus epidermidis*, and antibiotic susceptibility tests are performed to provide doctors with essential information for selecting the best drug for treatment of the infection. Antibacterial chemotherapy is initiated and delivered through the intravenous catheter that was originally inserted into the patient. Within 7 days, the skin infection is gone, blood cultures are negative for *S. epidermidis*, and the antibacterial chemotherapy is discontinued. However, 2 days after discontinuing the antibacterial chemotherapy, the patient develops another fever and skin infection and the blood cultures are positive for the same strain of *S. epidermidis* that had been isolated the previous week. This time, doctors remove the intravenous catheter and administer oral antibiotics, which successfully treat both the skin and blood-borne infection caused by *S. epidermidis*. Furthermore, the infection does not return after discontinuing the oral antibacterial chemotherapy. What are some possible reasons why intravenous chemotherapy failed to completely cure the patient despite laboratory tests showing the bacterial strain was susceptible to the prescribed antibiotic? Why might the second round of antibiotic therapy have been more successful? Justify your answers.

Exercise:

Problem: Why are autoinducers small molecules?

Exercise:**Problem:**

Refer to [Figure B1](#) in [Appendix B](#). If the results from a pond water sample were recorded as 3, 2, 1, what would be the MPN of bacteria in 100 mL of pond water?

Exercise:**Problem:**

Refer to [\[link\]](#). Why does turbidity lose reliability at high cell concentrations when the culture reaches the stationary phase?

Oxygen Requirements for Microbial Growth

LEARNING OBJECTIVES

- Interpret visual data demonstrating minimum, optimum, and maximum oxygen or carbon dioxide requirements for growth
- Identify and describe different categories of microbes with requirements for growth with or without oxygen: obligate aerobe, obligate anaerobe, facultative anaerobe, aerotolerant anaerobe, microaerophile, and capnophile
- Give examples of microorganisms for each category of growth requirements

Ask most people “What are the major requirements for life?” and the answers are likely to include water and oxygen. Few would argue about the need for water, but what about oxygen? Can there be life without oxygen?

The answer is that molecular oxygen (O_2) is not always needed. The earliest signs of life are dated to a period when conditions on earth were highly reducing and free oxygen gas was essentially nonexistent. Only after cyanobacteria started releasing oxygen as a byproduct of photosynthesis and the capacity of iron in the oceans for taking up oxygen was exhausted did oxygen levels increase in the atmosphere. This event, often referred to as the Great Oxygenation Event or the Oxygen Revolution, caused a massive extinction. Most organisms could not survive the powerful oxidative properties of **reactive oxygen species** (ROS), highly unstable ions and molecules derived from partial reduction of oxygen that can damage virtually any macromolecule or structure with which they come in contact. Singlet oxygen ($O_2^\bullet$), superoxide (O_2^-), peroxides (H_2O_2), hydroxyl

radical ($\text{OH}\bullet$), and hypochlorite ion (OCl^-), the active ingredient of household bleach, are all examples of ROS. The organisms that were able to detoxify reactive oxygen species harnessed the high electronegativity of oxygen to produce free energy for their metabolism and thrived in the new environment.

Oxygen Requirements of Microorganisms

Many ecosystems are still free of molecular oxygen. Some are found in extreme locations, such as deep in the ocean or in earth's crust; others are part of our everyday landscape, such as marshes, bogs, and sewers. Within the bodies of humans and other animals, regions with little or no oxygen provide an anaerobic environment for microorganisms. ([link](#)).



(a)



(b)

Anaerobic environments are still common on earth. They include environments like (a) a bog where undisturbed dense sediments are virtually devoid of oxygen, and (b) the rumen (the first compartment of a cow's stomach), which provides an oxygen-free incubator for methanogens and other obligate anaerobic bacteria. (credit a: modification of work by National Park Service; credit b: modification of work by US Department of Agriculture)

We can easily observe different requirements for molecular oxygen by growing bacteria in **thioglycolate tube cultures**. A test-tube culture starts with autoclaved **thioglycolate medium** containing a low percentage of agar to allow motile bacteria to move throughout the medium. Thioglycolate has strong reducing properties and autoclaving flushes out most of the oxygen. The tubes are inoculated with the bacterial cultures to be tested and incubated at an appropriate temperature. Over time, oxygen slowly diffuses throughout the thioglycolate tube culture from the top. Bacterial density increases in the area where oxygen concentration is best suited for the growth of that particular organism.

The growth of bacteria with varying oxygen requirements in thioglycolate tubes is illustrated in [\[link\]](#). In tube A, all the growth is seen at the top of the tube. The bacteria are **obligate (strict) aerobes** that cannot grow without an abundant supply of oxygen. Tube B looks like the opposite of tube A. Bacteria grow at the bottom of tube B. Those are **obligate anaerobes**, which are killed by oxygen. Tube C shows heavy growth at the top of the tube and growth throughout the tube, a typical result with **facultative anaerobes**. Facultative anaerobes are organisms that thrive in the presence of oxygen but also grow in its absence by relying on fermentation or anaerobic respiration, if there is a suitable electron acceptor other than oxygen and the organism is able to perform anaerobic respiration. The **aerotolerant anaerobes** in tube D are indifferent to the presence of oxygen. They do not use oxygen because they usually have a fermentative metabolism, but they are not harmed by the presence of oxygen as obligate anaerobes are. Tube E on the right shows a “Goldilocks” culture. The oxygen level has to be just right for growth, not too much and not too little. These **microaerophiles** are bacteria that require a minimum level of oxygen for growth, about 1%–10%, well below the 21% found in the atmosphere.

Examples of obligate aerobes are *Mycobacterium tuberculosis*, the causative agent of tuberculosis and *Micrococcus luteus*, a gram-positive bacterium that colonizes the skin. *Neisseria meningitidis*, the causative agent of severe bacterial meningitis, and *N. gonorrhoeae*, the causative agent of sexually transmitted gonorrhea, are also obligate aerobes.

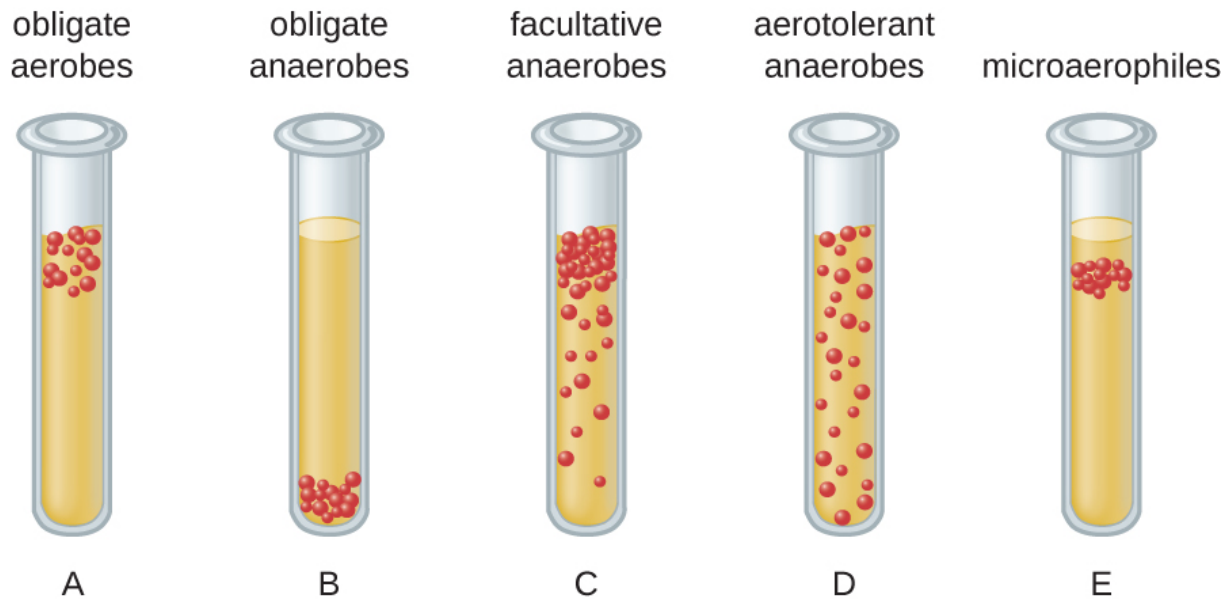


Diagram of bacterial cell distribution in thioglycolate tubes.

Many obligate anaerobes are found in the environment where anaerobic conditions exist, such as in deep sediments of soil, still waters, and at the bottom of the deep ocean where there is no photosynthetic life. Anaerobic conditions also exist naturally in the intestinal tract of animals. Obligate anaerobes, mainly *Bacteroidetes*, represent a large fraction of the microbes in the human gut. Transient anaerobic conditions exist when tissues are not supplied with blood circulation; they die and become an ideal breeding ground for obligate anaerobes. Another type of obligate anaerobe encountered in the human body is the gram-positive, rod-shaped *Clostridium* spp. Their ability to form endospores allows them to survive in the presence of oxygen. One of the major causes of health-acquired infections is *C. difficile*, known as *C. diff*. Prolonged use of antibiotics for other infections increases the probability of a patient developing a secondary *C. difficile* infection. Antibiotic treatment disrupts the balance of microorganisms in the intestine and allows the colonization of the gut by *C. difficile*, causing a significant inflammation of the colon.

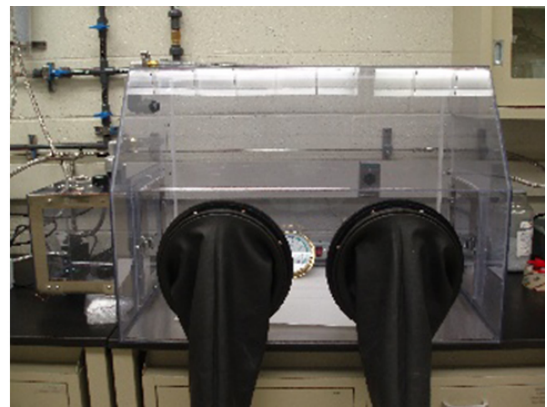
Other clostridia responsible for serious infections include *C. tetani*, the agent of tetanus, and *C. perfringens*, which causes gas gangrene. In both

cases, the infection starts in necrotic tissue (dead tissue that is not supplied with oxygen by blood circulation). This is the reason that deep puncture wounds are associated with tetanus. When tissue death is accompanied by lack of circulation, gangrene is always a danger.

The study of obligate anaerobes requires special equipment. Obligate anaerobic bacteria must be grown under conditions devoid of oxygen. The most common approach is culture in an **anaerobic jar** ([\[link\]](#)). Anaerobic jars include chemical packs that remove oxygen and release carbon dioxide (CO_2). An **anaerobic chamber** is an enclosed box from which all oxygen is removed. Gloves sealed to openings in the box allow handling of the cultures without exposing the culture to air ([\[link\]](#)).



(a)



(b)

(a) An anaerobic jar is pictured that is holding nine Petri plates supporting cultures. (b) Openings in the side of an anaerobic box are sealed by glove-like sleeves that allow for the handling of cultures inside the box. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by NIST)

Staphylococci and Enterobacteriaceae are examples of facultative anaerobes. Staphylococci are found on the skin and upper respiratory tract. Enterobacteriaceae are found primarily in the gut and upper respiratory tract

but can sometimes spread to the urinary tract, where they are capable of causing infections. It is not unusual to see mixed bacterial infections in which the facultative anaerobes use up the oxygen, creating an environment for the obligate anaerobes to flourish.

Examples of aerotolerant anaerobes include lactobacilli and streptococci, both found in the oral microbiota. *Campylobacter jejuni*, which causes gastrointestinal infections, is an example of a microaerophile and is grown under low-oxygen conditions.

The **optimum oxygen concentration**, as the name implies, is the ideal concentration of oxygen for a particular microorganism. The lowest concentration of oxygen that allows growth is called the **minimum permissive oxygen concentration**. The highest tolerated concentration of oxygen is the **maximum permissive oxygen concentration**. The organism will not grow outside the range of oxygen levels found between the minimum and maximum permissive oxygen concentrations.

Note:

- Would you expect the oldest bacterial lineages to be aerobic or anaerobic?
- Which bacteria grow at the top of a thioglycolate tube, and which grow at the bottom of the tube?

Note:

An Unwelcome Anaerobe

Charles is a retired bus driver who developed type 2 diabetes over 10 years ago. Since his retirement, his lifestyle has become very sedentary and he has put on a substantial amount of weight. Although he has felt tingling and numbness in his left foot for a while, he has not been worried because he thought his foot was simply “falling asleep.” Recently, a scratch on his foot does not seem to be healing and is becoming increasingly ugly.

Because the sore did not bother him much, Charles figured it could not be serious until his daughter noticed a purplish discoloration spreading on the skin and oozing ([link](#)). When he was finally seen by his physician, Charles was rushed to the operating room. His open sore, or ulcer, is the result of a diabetic foot.

The concern here is that gas gangrene may have taken hold in the dead tissue. The most likely agent of gas gangrene is *Clostridium perfringens*, an endospore-forming, gram-positive bacterium. It is an obligate anaerobe that grows in tissue devoid of oxygen. Since dead tissue is no longer supplied with oxygen by the circulatory system, the dead tissue provides pockets of ideal environment for the growth of *C. perfringens*.

A surgeon examines the ulcer and radiographs of Charles's foot and determines that the bone is not yet infected. The wound will have to be surgically debrided (debridement refers to the removal of dead and infected tissue) and a sample sent for microbiological lab analysis, but Charles will not have to have his foot amputated. Many diabetic patients are not so lucky. In 2008, nearly 70,000 diabetic patients in the United States lost a foot or limb to amputation, according to statistics from the Centers for Disease Control and Prevention.[\[footnote\]](#)

Centers for Disease Control and Prevention. "Living With Diabetes: Keep Your Feet Healthy." <http://www.cdc.gov/Features/DiabetesFootHealth/>

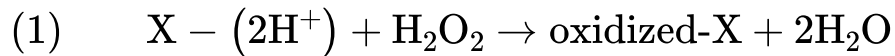
- Which growth conditions would you recommend for the detection of *C. perfringens*?



This clinical photo depicts ulcers on the foot of a diabetic patient. Dead tissue accumulating in ulcers can provide an ideal growth environment for the anaerobe *C. perfringens*, a causative agent of gas gangrene. (credit: Shigeo Kono, Reiko Nakagawachi, Jun Arata, Benjamin A Lipsky)

Detoxification of Reactive Oxygen Species

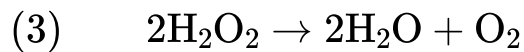
Aerobic respiration constantly generates reactive oxygen species (ROS), byproducts that must be detoxified. Even organisms that do not use aerobic respiration need some way to break down some of the ROS that may form from atmospheric oxygen. Three main enzymes break down those toxic byproducts: superoxide dismutase, peroxidase, and catalase. Each one catalyzes a different reaction. Reactions of type seen in Reaction 1 are catalyzed by **peroxidases**.

Equation:

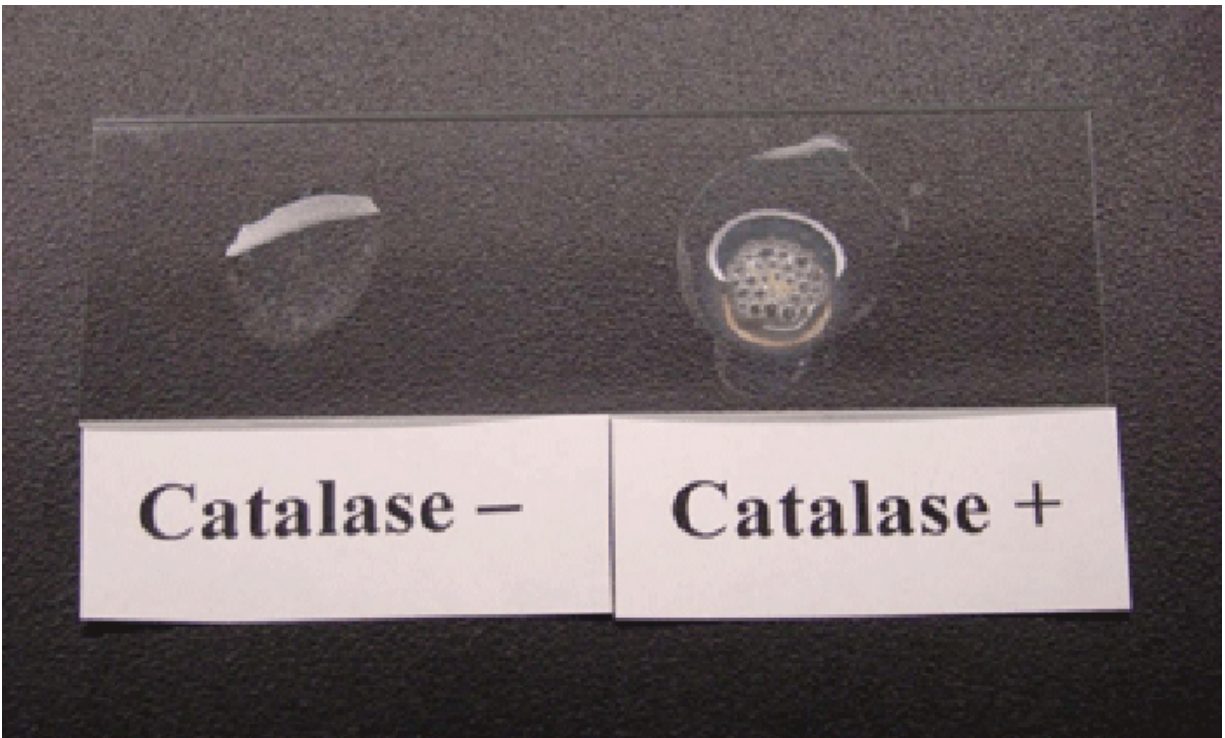
In these reactions, an electron donor (reduced compound; e.g., reduced nicotinamide adenine dinucleotide [NADH]) oxidizes hydrogen peroxide, or other peroxides, to water. The enzymes play an important role by limiting the damage caused by peroxidation of membrane lipids. Reaction 2 is mediated by the enzyme **superoxide dismutase** (SOD) and breaks down the powerful superoxide anions generated by aerobic metabolism:

Equation:

The enzyme **catalase** converts hydrogen peroxide to water and oxygen as shown in Reaction 3.

Equation:

Obligate anaerobes usually lack all three enzymes. Aerotolerant anaerobes do have SOD but no catalase. Reaction 3, shown occurring in [\[link\]](#), is the basis of a useful and rapid test to distinguish streptococci, which are aerotolerant and do not possess catalase, from staphylococci, which are facultative anaerobes. A sample of culture rapidly mixed in a drop of 3% hydrogen peroxide will release bubbles if the culture is catalase positive.



The catalase test detects the presence of the enzyme catalase by noting whether bubbles are released when hydrogen peroxide is added to a culture sample. Compare the positive result (right) with the negative result (left). (credit: Centers for Disease Control and Prevention)

Bacteria that grow best in a higher concentration of CO_2 and a lower concentration of oxygen than present in the atmosphere are called **capnophiles**. One common approach to grow capnophiles is to use a **candle jar**. A candle jar consists of a jar with a tight-fitting lid that can accommodate the cultures and a candle. After the cultures are added to the jar, the candle is lit and the lid closed. As the candle burns, it consumes most of the oxygen present and releases CO_2 .

Note:

- What substance is added to a sample to detect catalase?

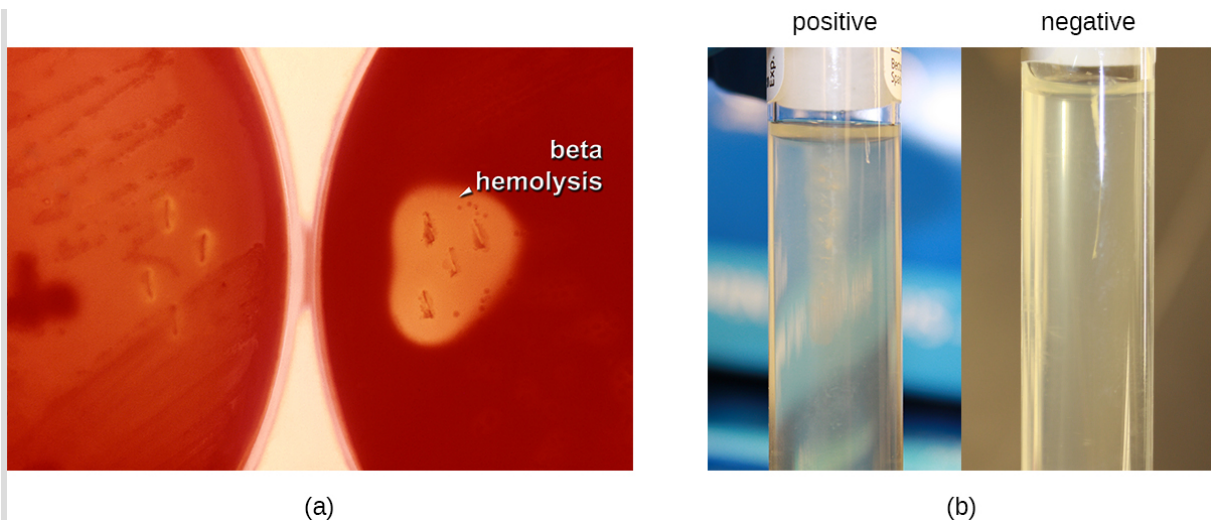
- What is the function of the candle in a candle jar?

Note:

Part 2

The health-care provider who saw Jeni was concerned primarily because of her pregnancy. Her condition enhances the risk for infections and makes her more vulnerable to those infections. The immune system is downregulated during pregnancy, and pathogens that cross the placenta can be very dangerous for the fetus. A note on the provider's order to the microbiology lab mentions a suspicion of infection by *Listeria monocytogenes*, based on the signs and symptoms exhibited by the patient. Jeni's blood samples are streaked directly on sheep blood agar, a medium containing tryptic soy agar enriched with 5% sheep blood. (Blood is considered sterile; therefore, competing microorganisms are not expected in the medium.) The inoculated plates are incubated at 37 °C for 24 to 48 hours. Small grayish colonies surrounded by a clear zone emerge. Such colonies are typical of *Listeria* and other pathogens such as streptococci; the clear zone surrounding the colonies indicates complete lysis of blood in the medium, referred to as beta-hemolysis ([\[link\]](#)). When tested for the presence of catalase, the colonies give a positive response, eliminating *Streptococcus* as a possible cause. Furthermore, a Gram stain shows short gram-positive bacilli. Cells from a broth culture grown at room temperature displayed the tumbling motility characteristic of *Listeria* ([\[link\]](#)). All of these clues lead the lab to positively confirm the presence of *Listeria* in Jeni's blood samples.

- How serious is Jeni's condition and what is the appropriate treatment?



(a) A sample blood agar test showing beta-hemolysis. (b) A sample motility test showing both positive and negative results. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by “VeeDunn”/Flickr)

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Aerobic and anaerobic environments can be found in diverse niches throughout nature, including different sites within and on the human body.
- Microorganisms vary in their requirements for molecular oxygen. **Obligate aerobes** depend on aerobic respiration and use oxygen as a terminal electron acceptor. They cannot grow without oxygen.
- **Obligate anaerobes** cannot grow in the presence of oxygen. They depend on fermentation and anaerobic respiration using a final electron acceptor other than oxygen.
- **Facultative anaerobes** show better growth in the presence of oxygen but will also grow without it.

- Although **aerotolerant anaerobes** do not perform aerobic respiration, they can grow in the presence of oxygen. Most aerotolerant anaerobes test negative for the enzyme **catalase**.
- **Microaerophiles** need oxygen to grow, albeit at a lower concentration than 21% oxygen in air.
- **Optimum oxygen concentration** for an organism is the oxygen level that promotes the fastest growth rate. The **minimum permissive oxygen concentration** and the **maximum permissive oxygen concentration** are, respectively, the lowest and the highest oxygen levels that the organism will tolerate.
- **Peroxidase, superoxide dismutase, and catalase** are the main enzymes involved in the detoxification of the **reactive oxygen species**. Superoxide dismutase is usually present in a cell that can tolerate oxygen. All three enzymes are usually detectable in cells that perform aerobic respiration and produce more ROS.
- A **capnophile** is an organism that requires a higher than atmospheric concentration of CO₂ to grow.

Multiple Choice

Exercise:

Problem:

An inoculated thioglycolate medium culture tube shows dense growth at the surface and turbidity throughout the rest of the tube. What is your conclusion?

- A. The organisms die in the presence of oxygen
- B. The organisms are facultative anaerobes.
- C. The organisms should be grown in an anaerobic chamber.
- D. The organisms are obligate aerobes.

Solution:

B

Exercise:

Problem:

An inoculated thioglycolate medium culture tube is clear throughout the tube except for dense growth at the bottom of the tube. What is your conclusion?

- A. The organisms are obligate anaerobes.
- B. The organisms are facultative anaerobes.
- C. The organisms are aerotolerant.
- D. The organisms are obligate aerobes.

Solution:

A

Exercise:

Problem:

Pseudomonas aeruginosa is a common pathogen that infects the airways of patients with cystic fibrosis. It does not grow in the absence of oxygen. The bacterium is probably which of the following?

- A. an aerotolerant anaerobe
- B. an obligate aerobe
- C. an obligate anaerobe
- D. a facultative anaerobe

Solution:

B

Exercise:

Problem:

Streptococcus mutans is a major cause of cavities. It resides in the gum pockets, does not have catalase activity, and can be grown outside of an anaerobic chamber. The bacterium is probably which of the following?

- A. a facultative anaerobe
 - B. an obligate aerobe
 - C. an obligate anaerobe
 - D. an aerotolerant anaerobe
-

Solution:

D

Exercise:**Problem:**

Why do the instructions for the growth of *Neisseria gonorrhoeae* recommend a CO₂-enriched atmosphere?

- A. It uses CO₂ as a final electron acceptor in respiration.
 - B. It is an obligate anaerobe.
 - C. It is a capnophile.
 - D. It fixes CO₂ through photosynthesis.
-

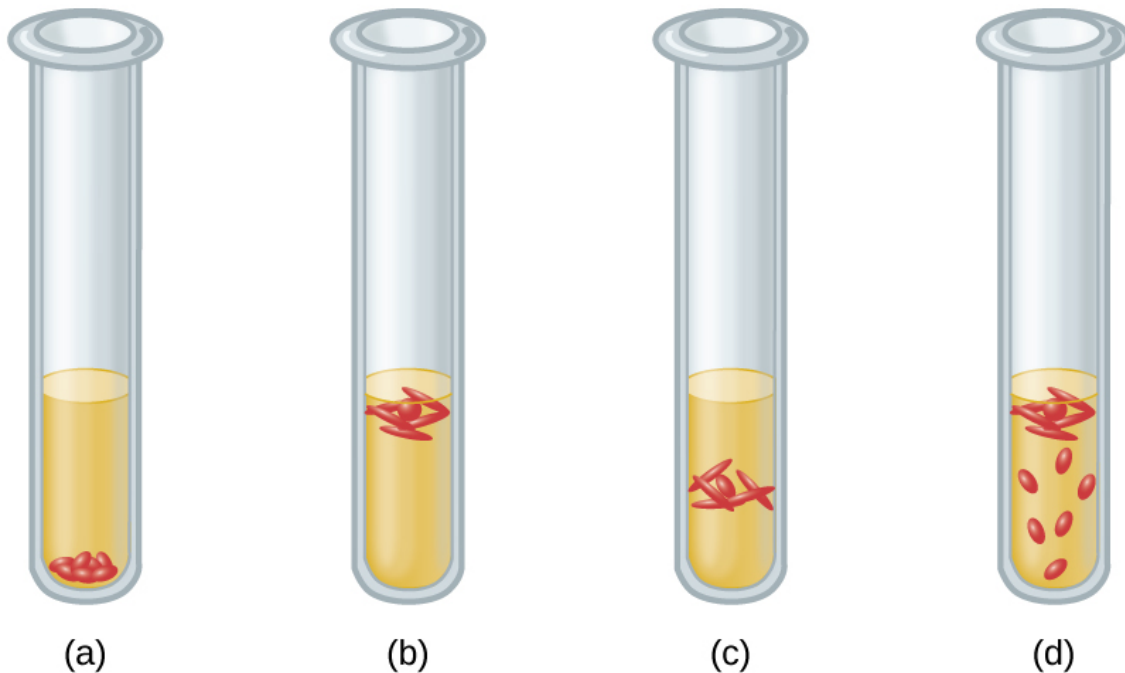
Solution:

C

Matching**Exercise:**

Problem:

Four tubes are illustrated with cultures grown in a medium that slows oxygen diffusion. Match the culture tube with the correct type of bacteria from the following list: facultative anaerobe, obligate anaerobe, microaerophile, aerotolerant anaerobe, obligate aerobe.



Solution:

(a) obligate anaerobe, (b) obligate aerobe, (c) microaerophile, (d) facultative anaerobe

Short Answer**Exercise:****Problem:**

Why are some obligate anaerobes able to grow in tissues (e.g., gum pockets) that are not completely free of oxygen?

Exercise:**Problem:**

Why should *Haemophilus influenzae* be grown in a candle jar?

Exercise:**Problem:**

In terms of oxygen requirements, what type of organism would most likely be responsible for a foodborne illness associated with canned foods?

Critical Thinking**Exercise:****Problem:**

A microbiology instructor prepares cultures for a gram-staining practical laboratory by inoculating growth medium with a gram-positive coccus (nonmotile) and a gram-negative rod (motile). The goal is to demonstrate staining of a mixed culture. The flask is incubated at 35 °C for 24 hours without aeration. A sample is stained and reveals only gram-negative rods. Both cultures are known facultative anaerobes. Give a likely reason for success of the gram-negative rod. Assume that the cultures have comparable intrinsic growth rates.

The Effects of pH on Microbial Growth

LEARNING OBJECTIVES

- Illustrate and briefly describe minimum, optimum, and maximum pH requirements for growth
- Identify and describe the different categories of microbes with pH requirements for growth: acidophiles, neutrophiles, and alkaliphiles
- Give examples of microorganisms for each category of pH requirement

Yogurt, pickles, sauerkraut, and lime-seasoned dishes all owe their tangy taste to a high acid content ([\[link\]](#)). Recall that acidity is a function of the concentration of hydrogen ions $[H^+]$ and is measured as pH. Environments with pH values below 7.0 are considered acidic, whereas those with pH values above 7.0 are considered basic. Extreme pH affects the structure of all macromolecules. The hydrogen bonds holding together strands of DNA break up at high pH. Lipids are hydrolyzed by an extremely basic pH. The proton motive force responsible for production of ATP in cellular respiration depends on the concentration gradient of H^+ across the plasma membrane (see [Cellular Respiration](#)). If H^+ ions are neutralized by hydroxide ions, the concentration gradient collapses and impairs energy production. But the component most sensitive to pH in the cell is its workhorse, the protein. Moderate changes in pH modify the ionization of amino-acid functional groups and disrupt hydrogen bonding, which, in turn, promotes changes in the folding of the molecule, promoting denaturation and destroying activity.



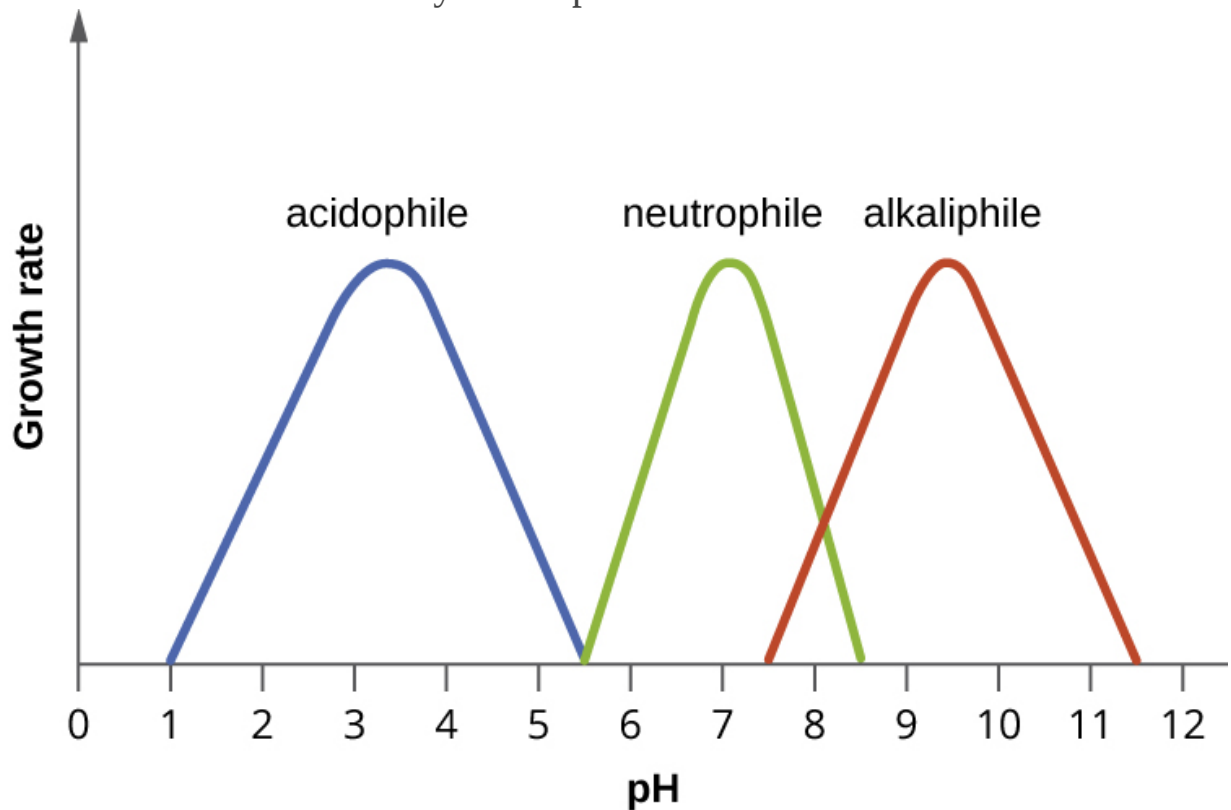
Lactic acid bacteria that ferment milk into yogurt or transform vegetables in pickles thrive at a pH close to 4.0. Sauerkraut and dishes such as pico de gallo owe their tangy flavor to their acidity. Acidic foods have been a mainstay of the human diet for centuries, partly because most microbes that cause food spoilage grow best at a near neutral pH and do not tolerate acidity well. (credit “yogurt”: modification of work by “nina.jsc”/Flickr; credit “pickles”: modification of work by Noah Sussman; credit “sauerkraut”: modification of work by Jesse LaBuff; credit “pico de gallo”: modification of work by “regan76”/Flickr)

The **optimum growth pH** is the most favorable pH for the growth of an organism. The lowest pH value that an organism can tolerate is called the **minimum growth pH** and the highest pH is the **maximum growth pH**. These values can cover a wide range, which is important for the preservation of food and to microorganisms’ survival in the stomach. For example, the optimum growth pH of *Salmonella* spp. is 7.0–7.5, but the minimum growth pH is closer to 4.2.

Most bacteria are **neutrophiles**, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7 (see [\[link\]](#)). Most familiar bacteria, like *Escherichia coli*, staphylococci, and *Salmonella* spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli*, *S. typhi*, and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0–6.0.

Microorganisms that grow optimally at pH less than 5.55 are called **acidophiles**. For example, the sulfur-oxidizing *Sulfolobus* spp. isolated

from sulfur mud fields and hot springs in Yellowstone National Park are extreme acidophiles. These archaea survive at pH values of 2.5–3.5. Species of the archaean genus *Ferroplasma* live in acid mine drainage at pH values of 0–2.9. *Lactobacillus* bacteria, which are an important part of the normal microbiota of the vagina, can tolerate acidic environments at pH values 3.5–6.8 and also contribute to the acidity of the vagina (pH of 4, except at the onset of menstruation) through their metabolic production of lactic acid. The vagina's acidity plays an important role in inhibiting other microbes that are less tolerant of acidity. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H^+ ions out of the cells. The changes in the composition of membrane phospholipids probably reflect the need to maintain membrane fluidity at low pH.



The curves show the approximate pH ranges for the growth of the different classes of pH-specific prokaryotes. Each curve has an optimal pH and extreme pH values at which growth is much reduced. Most bacteria are neutrophiles and grow best at near-neutral pH (center

curve). Acidophiles have optimal growth at pH values near 3 and alkaliphiles have optimal growth at pH values above 9.

At the other end of the spectrum are **alkaliphiles**, microorganisms that grow best at pH between 8.0 and 10.5. *Vibrio cholerae*, the pathogenic agent of cholera, grows best at the slightly basic pH of 8.0; it can survive pH values of 11.0 but is inactivated by the acid of the stomach. When it comes to survival at high pH, the bright pink archaean *Natronobacterium*, found in the soda lakes of the African Rift Valley, may hold the record at a pH of 10.5 ([link](#)). Extreme alkaliphiles have adapted to their harsh environment through evolutionary modification of lipid and protein structure and compensatory mechanisms to maintain the proton motive force in an alkaline environment. For example, the alkaliphile *Bacillus firmus* derives the energy for transport reactions and motility from a Na^+ ion gradient rather than a proton motive force. Many enzymes from alkaliphiles have a higher isoelectric point, due to an increase in the number of basic amino acids, than homologous enzymes from neutrophiles.



View from space of Lake Natron in Tanzania. The pink color is due to the pigmentation of the extreme alkaliphilic and halophilic microbes that colonize the lake. (credit: NASA)

Note:

Survival at the Low pH of the Stomach

Peptic ulcers (or stomach ulcers) are painful sores on the stomach lining. Until the 1980s, they were believed to be caused by spicy foods, stress, or a combination of both. Patients were typically advised to eat bland foods, take anti-acid medications, and avoid stress. These remedies were not particularly effective, and the condition often recurred. This all changed dramatically when the real cause of most peptic ulcers was discovered to be a slim, corkscrew-shaped bacterium, *Helicobacter pylori*. This organism was identified and isolated by Barry Marshall and Robin Warren, whose discovery earned them the Nobel Prize in Medicine in 2005.

The ability of *H. pylori* to survive the low pH of the stomach would seem to suggest that it is an extreme acidophile. As it turns out, this is not the case. In fact, *H. pylori* is a neutrophile. So, how does it survive in the stomach? Remarkably, *H. pylori* creates a microenvironment in which the pH is nearly neutral. It achieves this by producing large amounts of the enzyme urease, which breaks down urea to form NH_4^+ and CO_2 . The ammonium ion raises the pH of the immediate environment.

This metabolic capability of *H. pylori* is the basis of an accurate, noninvasive test for infection. The patient is given a solution of urea containing radioactively labeled carbon atoms. If *H. pylori* is present in the stomach, it will rapidly break down the urea, producing radioactive CO_2 that can be detected in the patient's breath. Because peptic ulcers may lead to gastric cancer, patients who are determined to have *H. pylori* infections are treated with antibiotics.

Note:

- What effect do extremes of pH have on proteins?
- What pH-adaptive type of bacteria would most human pathogens be?

Key Concepts and Summary

- Bacteria are generally **neutrophiles**. They grow best at neutral pH close to 7.0.
- **Acidophiles** grow optimally at a pH near 3.0. **Alkaliphiles** are organisms that grow optimally between a pH of 8 and 10.5. Extreme acidophiles and alkaliphiles grow slowly or not at all near neutral pH.
- Microorganisms grow best at their **optimum growth pH**. Growth occurs slowly or not at all below the **minimum growth pH** and above the **maximum growth pH**.

Multiple Choice

Exercise:

Problem:

Bacteria that grow in mine drainage at pH 1–2 are probably which of the following?

- A. alkaliphiles
- B. acidophiles
- C. neutrophiles
- D. obligate anaerobes

Solution:

B

Exercise:

Problem:

Bacteria isolated from Lake Natron, where the water pH is close to 10, are which of the following?

- A. alkaliphiles
- B. facultative anaerobes
- C. neutrophiles
- D. obligate anaerobes

Solution:

A

Exercise:**Problem:**

In which environment are you most likely to encounter an acidophile?

- A. human blood at pH 7.2
- B. a hot vent at pH 1.5
- C. human intestine at pH 8.5
- D. milk at pH 6.5

Solution:

B

Fill in the Blank**Exercise:****Problem:**

A bacterium that thrives in a soda lake where the average pH is 10.5 can be classified as a(n) _____.

Solution:

alkaliphile

Exercise:**Problem:**

Lactobacillus acidophilus grows best at pH 4.5. It is considered a(n) _____.

Solution:

acidophile

Short Answer**Exercise:****Problem:**

Which macromolecule in the cell is most sensitive to changes in pH?

Exercise:**Problem:**

Which metabolic process in the bacterial cell is particularly challenging at high pH?

Critical Thinking**Exercise:****Problem:**

People who use proton pumps inhibitors or antacids are more prone to infections of the gastrointestinal tract. Can you explain the observation in light of what you have learned?

Temperature and Microbial Growth

LEARNING OBJECTIVES

- Illustrate and briefly describe minimum, optimum, and maximum temperature requirements for growth
- Identify and describe different categories of microbes with temperature requirements for growth: psychrophile, psychrotrophs, mesophile, thermophile, hyperthermophile
- Give examples of microorganisms in each category of temperature tolerance

When the exploration of Lake Whillans started in Antarctica, researchers did not expect to find much life. Constant subzero temperatures and lack of obvious sources of nutrients did not seem to be conditions that would support a thriving ecosystem. To their surprise, the samples retrieved from the lake showed abundant microbial life. In a different but equally harsh setting, bacteria grow at the bottom of the ocean in sea vents ([\[link\]](#)), where temperatures can reach 340 °C (700 °F).

Microbes can be roughly classified according to the range of temperature at which they can grow. The growth rates are the highest at the **optimum growth temperature** for the organism. The lowest temperature at which the organism can survive and replicate is its **minimum growth temperature**. The highest temperature at which growth can occur is its **maximum growth temperature**. The following ranges of permissive growth temperatures are approximate only and can vary according to other environmental factors.

Organisms categorized as **mesophiles** (“middle loving”) are adapted to moderate temperatures, with optimal growth temperatures ranging from room temperature (about 20 °C) to about 45 °C. As would be expected from the core temperature of the human body, 37 °C (98.6 °F), normal human microbiota and pathogens (e.g., *E. coli*, *Salmonella* spp., and *Lactobacillus* spp.) are mesophiles.

Organisms called **psychrotrophs**, also known as psychrotolerant, prefer cooler environments, from a high temperature of 25 °C to refrigeration temperature about 4 °C. They are found in many natural environments in temperate climates. They are also responsible for the spoilage of refrigerated food.

Note:

Resolution

The presence of *Listeria* in Jeni’s blood suggests that her symptoms are due to listeriosis, an infection caused by *L. monocytogenes*. Listeriosis is a serious infection with a 20% mortality rate and is a particular risk to Jeni’s fetus. A sample from the amniotic fluid cultured for the presence of *Listeria* gave negative results. Because the absence of organisms does not rule out the possibility of infection, a molecular test based on the nucleic acid amplification of the 16S ribosomal RNA of *Listeria* was performed to confirm that no bacteria crossed the placenta. Fortunately, the results from the molecular test were also negative.

Jeni was admitted to the hospital for treatment and recovery. She received a high dose of two antibiotics intravenously for 2 weeks. The preferred drugs for the treatment of listeriosis are ampicillin or penicillin G with an aminoglycoside antibiotic. Resistance to common antibiotics is still rare in *Listeria* and antibiotic treatment is usually successful. She was released to home care after a week and fully recovered from her infection.

L. monocytogenes is a gram-positive short rod found in soil, water, and food. It is classified as a psychrophile and is halotolerant. Its ability to multiply at refrigeration temperatures (4–10 °C) and its tolerance for high concentrations of salt (up to 10% sodium chloride [NaCl]) make it a frequent source of food poisoning. Because *Listeria* can infect animals, it

often contaminates food such as meat, fish, or dairy products. Contamination of commercial foods can often be traced to persistent biofilms that form on manufacturing equipment that is not sufficiently cleaned.

Listeria infection is relatively common among pregnant women because the elevated levels of progesterone downregulate the immune system, making them more vulnerable to infection. The pathogen can cross the placenta and infect the fetus, often resulting in miscarriage, stillbirth, or fatal neonatal infection. Pregnant women are thus advised to avoid consumption of soft cheeses, refrigerated cold cuts, smoked seafood, and unpasteurized dairy products. Because *Listeria* bacteria can easily be confused with diphtheroids, another common group of gram-positive rods, it is important to alert the laboratory when listeriosis is suspected.

Go back to the [previous](#) Clinical Focus box.

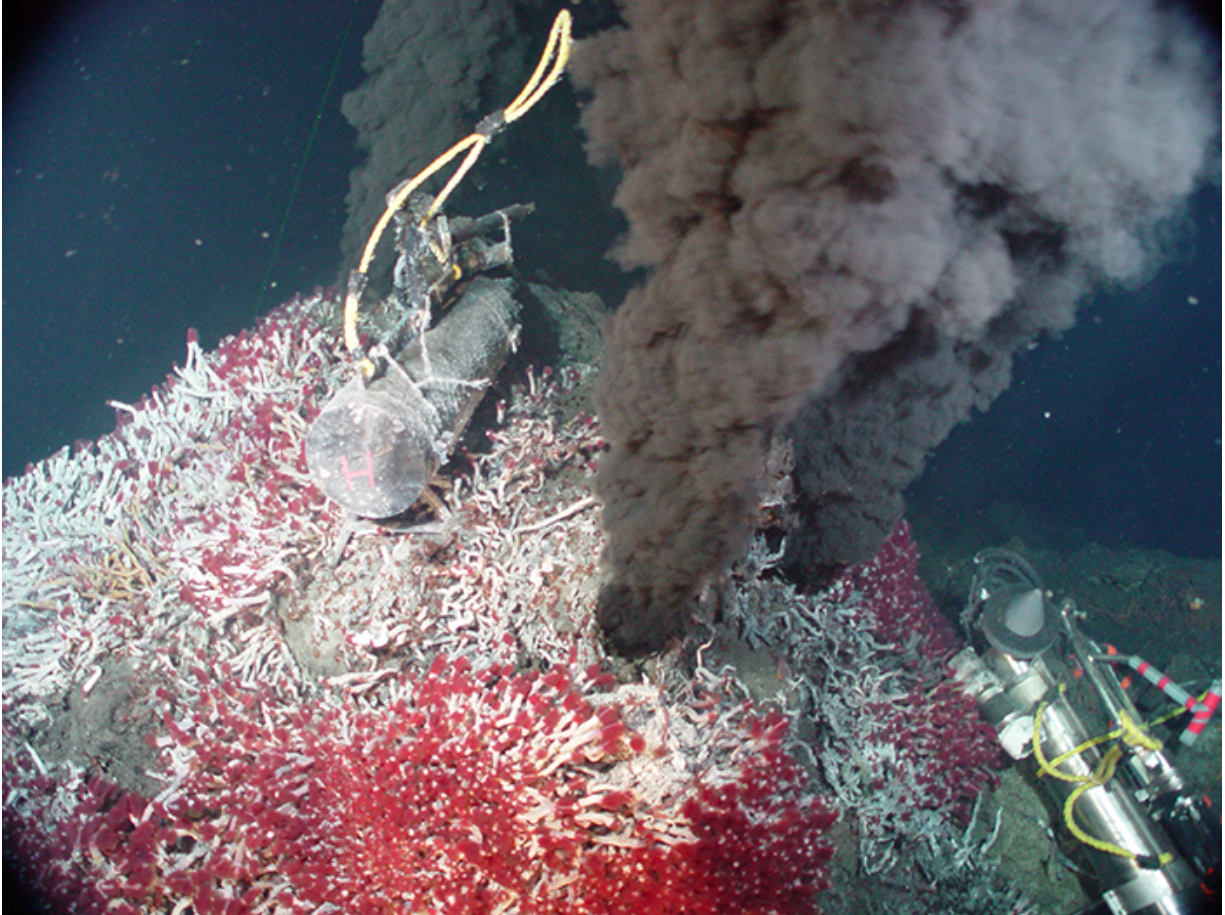
The organisms retrieved from arctic lakes such as Lake Whillans are considered extreme **psychrophiles** (cold loving). Psychrophiles are microorganisms that can grow at 0 °C and below, have an optimum growth temperature close to

15 °C, and usually do not survive at temperatures above 20 °C. They are found in permanently cold environments such as the deep waters of the oceans. Because they are active at low temperature, psychrophiles and psychrotrophs are important decomposers in cold climates.

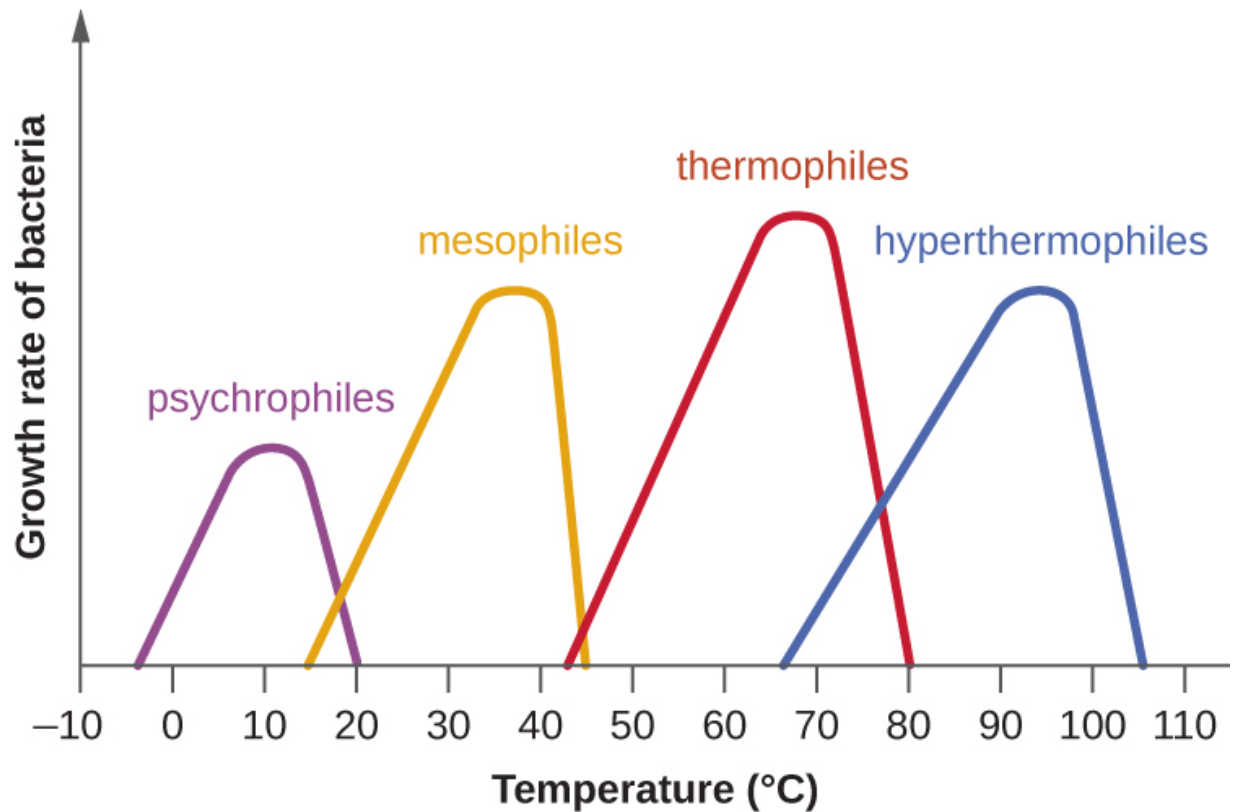
Organisms that grow at optimum temperatures of 50 °C to a maximum of 80 °C are called **thermophiles** (“heat loving”). They do not multiply at room temperature. Thermophiles are widely distributed in hot springs, geothermal soils, and manmade environments such as garden compost piles where the microbes break down kitchen scraps and vegetal material.

Examples of thermophiles include *Thermus aquaticus* and *Geobacillus* spp. Higher up on the extreme temperature scale we find the **hyperthermophiles**, which are characterized by growth ranges from 80 °C to a maximum of 110 °C, with some extreme examples that survive temperatures above 121 °C, the average temperature of an autoclave. The hydrothermal vents at the bottom of the ocean are a prime example of

extreme environments, with temperatures reaching an estimated 340 °C ([\[link\]](#)). Microbes isolated from the vents achieve optimal growth at temperatures higher than 100 °C. Noteworthy examples are *Pyrobolus* and *Pyrodictium*, archaea that grow at 105 °C and survive autoclaving. [\[link\]](#) shows the typical skewed curves of temperature-dependent growth for the categories of microorganisms we have discussed.



A black smoker at the bottom of the ocean belches hot, chemical-rich water, and heats the surrounding waters. Sea vents provide an extreme environment that is nonetheless teeming with macroscopic life (the red tubeworms) supported by an abundant microbial ecosystem. (credit: NOAA)



The graph shows growth rate of bacteria as a function of temperature. Notice that the curves are skewed toward the optimum temperature.

The skewing of the growth curve is thought to reflect the rapid denaturation of proteins as the temperature rises past the optimum for growth of the microorganism.

Life in extreme environments raises fascinating questions about the adaptation of macromolecules and metabolic processes. Very low temperatures affect cells in many ways. Membranes lose their fluidity and are damaged by ice crystal formation. Chemical reactions and diffusion slow considerably. Proteins become too rigid to catalyze reactions and may undergo denaturation. At the opposite end of the temperature spectrum, heat denatures proteins and nucleic acids. Increased fluidity impairs metabolic processes in membranes. Some of the practical applications of the destructive effects of heat on microbes are sterilization by steam, pasteurization, and incineration of inoculating loops. Proteins in

psychrophiles are, in general, rich in hydrophobic residues, display an increase in flexibility, and have a lower number of secondary stabilizing bonds when compared with homologous proteins from mesophiles.

Antifreeze proteins and solutes that decrease the freezing temperature of the cytoplasm are common. The lipids in the membranes tend to be unsaturated to increase fluidity. Growth rates are much slower than those encountered at moderate temperatures. Under appropriate conditions, mesophiles and even thermophiles can survive freezing. Liquid cultures of bacteria are mixed with sterile glycerol solutions and frozen to -80°C for long-term storage as stocks. Cultures can withstand freeze drying (lyophilization) and then be stored as powders in sealed ampules to be reconstituted with broth when needed.

Macromolecules in thermophiles and hyperthermophiles show some notable structural differences from what is observed in the mesophiles. The ratio of saturated to polyunsaturated lipids increases to limit the fluidity of the cell membranes. Their DNA sequences show a higher proportion of guanine–cytosine nitrogenous bases, which are held together by three hydrogen bonds in contrast to adenine and thymine, which are connected in the double helix by two hydrogen bonds. Additional secondary ionic and covalent bonds, as well as the replacement of key amino acids to stabilize folding, contribute to the resistance of proteins to denaturation. The so-called thermoenzymes purified from thermophiles have important practical applications. For example, amplification of nucleic acids in the polymerase chain reaction (PCR) depends on the thermal stability of *Taq* polymerase, an enzyme isolated from *T. aquaticus*. Degradation enzymes from thermophiles are added as ingredients in hot-water detergents, increasing their effectiveness.

Note:

- What temperature requirements do most bacterial human pathogens have?
- What DNA adaptation do thermophiles exhibit?

Note:**Feeding the World...and the World's Algae**

Artificial fertilizers have become an important tool in food production around the world. They are responsible for many of the gains of the so-called green revolution of the 20th century, which has allowed the planet to feed many of its more than 7 billion people. Artificial fertilizers provide nitrogen and phosphorus, key limiting nutrients, to crop plants, removing the normal barriers that would otherwise limit the rate of growth. Thus, fertilized crops grow much faster, and farms that use fertilizer produce higher crop yields.

However, careless use and overuse of artificial fertilizers have been demonstrated to have significant negative impacts on aquatic ecosystems, both freshwater and marine. Fertilizers that are applied at inappropriate times or in too-large quantities allow nitrogen and phosphorus compounds to escape use by crop plants and enter drainage systems. Inappropriate use of fertilizers in residential settings can also contribute to nutrient loads, which find their way to lakes and coastal marine ecosystems. As water warms and nutrients are plentiful, microscopic algae bloom, often changing the color of the water because of the high cell density.

Most algal blooms are not directly harmful to humans or wildlife; however, they can cause harm indirectly. As the algal population expands and then dies, it provides a large increase in organic matter to the bacteria that live in deep water. With this large supply of nutrients, the population of nonphotosynthetic microorganisms explodes, consuming available oxygen and creating “dead zones” where animal life has virtually disappeared.

Depletion of oxygen in the water is not the only damaging consequence of some algal blooms. The algae that produce red tides in the Gulf of Mexico, *Karenia brevis*, secrete potent toxins that can kill fish and other organisms and also accumulate in shellfish. Consumption of contaminated shellfish can cause severe neurological and gastrointestinal symptoms in humans. Shellfish beds must be regularly monitored for the presence of the toxins, and harvests are often shut down when it is present, incurring economic costs to the fishery. Cyanobacteria, which can form blooms in marine and freshwater ecosystems, produce toxins called microcystins, which can cause allergic reactions and liver damage when ingested in drinking water or during swimming. Recurring cyanobacterial algal blooms in Lake Erie

([link](#)) have forced municipalities to issue drinking water bans for days at a time because of unacceptable toxin levels.

This is just a small sampling of the negative consequences of algal blooms, red tides, and dead zones. Yet the benefits of crop fertilizer—the main cause of such blooms—are difficult to dispute. There is no easy solution to this dilemma, as a ban on fertilizers is not politically or economically feasible. In lieu of this, we must advocate for responsible use and regulation in agricultural and residential contexts, as well as the restoration of wetlands, which can absorb excess fertilizers before they reach lakes and oceans.



Heavy rains cause runoff of fertilizers into Lake Erie, triggering extensive algal blooms, which can be observed along the shoreline. Notice the brown unplanted and green planted agricultural land on the shore. (credit: NASA)

Note:

This [video](#) discusses algal blooms and dead zones in more depth.

Key Concepts and Summary

- Microorganisms thrive at a wide range of temperatures; they have colonized different natural environments and have adapted to extreme temperatures. Both extreme cold and hot temperatures require evolutionary adjustments to macromolecules and biological processes.
- **Psychrophiles** grow best in the temperature range of 0–15 °C whereas **psychrotrophs** thrive between 4°C and 25 °C.
- **Mesophiles** grow best at moderate temperatures in the range of 20 °C to about 45 °C. Pathogens are usually mesophiles.
- **Thermophiles** and **hyperthermophiles** are adapted to life at temperatures above 50 °C.
- Adaptations to cold and hot temperatures require changes in the composition of membrane lipids and proteins.

Multiple Choice

Exercise:**Problem:**

A soup container was forgotten in the refrigerator and shows contamination. The contaminants are probably which of the following?

- A. thermophiles
- B. acidophiles
- C. mesophiles
- D. psychrotrophs

Solution:

D

Exercise:

Problem:

Bacteria isolated from a hot tub at 39 °C are probably which of the following?

- A. thermophiles
- B. psychrotrophs
- C. mesophiles
- D. hyperthermophiles

Solution:

C

Exercise:

Problem:

In which environment are you most likely to encounter a hyperthermophile?

- A. hot tub
- B. warm ocean water in Florida
- C. hydrothermal vent at the bottom of the ocean
- D. human body

Solution:

C

Exercise:

Problem:

Which of the following environments would harbor psychrophiles?

- A. mountain lake with a water temperature of 12 °C

- B. contaminated plates left in a 35 °C incubator
- C. yogurt cultured at room temperature
- D. salt pond in the desert with a daytime temperature of 34 °C

Solution:

A

Matching

Exercise:

Problem:

Match the type of bacterium with its environment. Each choice may be used once, more than once, or not at all. Put the appropriate letter beside the environment.

___psychotroph	A. food spoiling in refrigerator
___mesophile	B. hydrothermal vent
___thermophile	C. deep ocean waters
___hyperthermophile	D. human pathogen
___psychrophile	E. garden compost

Solution:

A, D, E, B, C

Short Answer

Exercise:

Problem:

How are hyperthermophile's proteins adapted to the high temperatures of their environment?

Exercise:

Problem:

Why would NASA be funding microbiology research in Antarctica?

Critical Thinking

Exercise:

Problem:

The bacterium that causes Hansen's disease (leprosy), *Mycobacterium leprae*, infects mostly the extremities of the body: hands, feet, and nose. Can you make an educated guess as to its optimum temperature of growth?

Exercise:

Problem:

Refer to [\[link\]](#). Some hyperthermophiles can survive autoclaving temperatures. Are they a concern in health care?

Other Environmental Conditions that Affect Growth

LEARNING OBJECTIVES

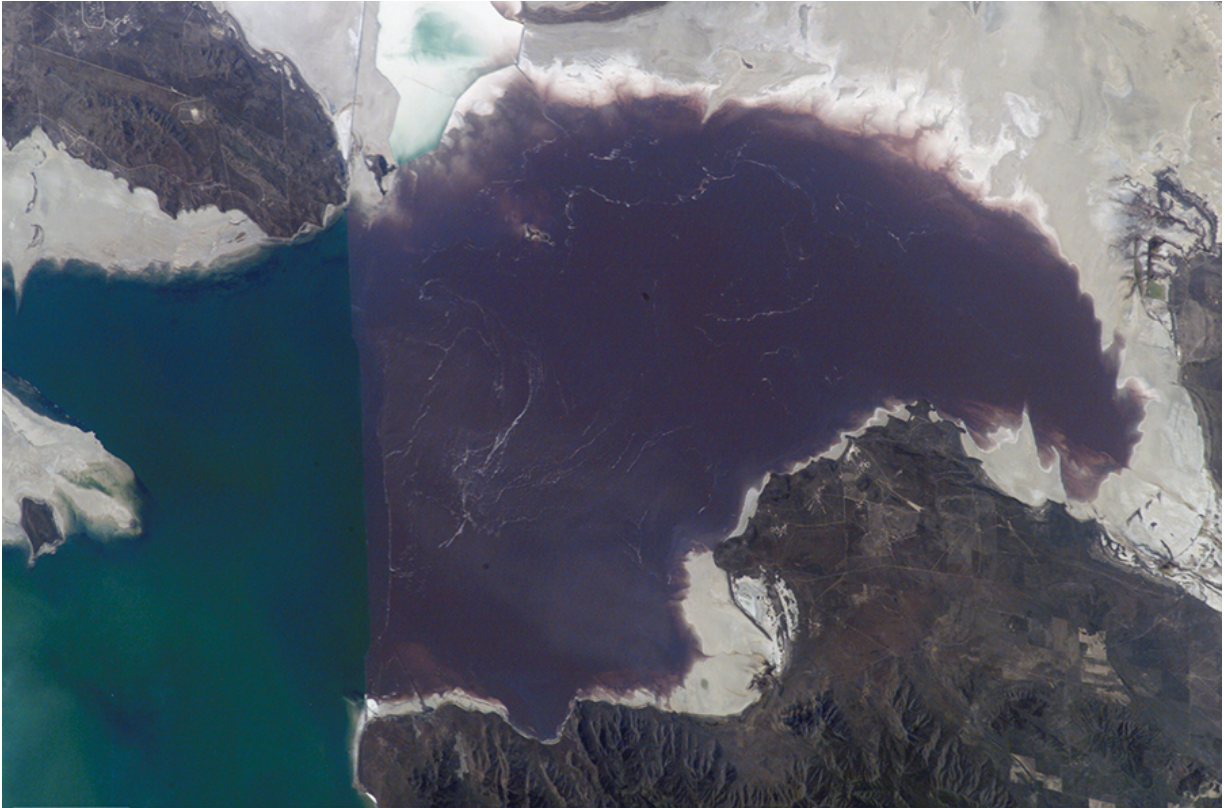
- Identify and describe different categories of microbes with specific growth requirements other than oxygen, pH, and temperature, such as altered barometric pressure, osmotic pressure, humidity, and light
- Give at least one example microorganism for each category of growth requirement

Microorganisms interact with their environment along more dimensions than pH, temperature, and free oxygen levels, although these factors require significant adaptations. We also find microorganisms adapted to varying levels of salinity, barometric pressure, humidity, and light.

Osmotic and Barometric Pressure

Most natural environments tend to have lower solute concentrations than the cytoplasm of most microorganisms. Rigid cell walls protect the cells from bursting in a dilute environment. Not much protection is available against high osmotic pressure. In this case, water, following its concentration gradient, flows out of the cell. This results in plasmolysis (the shrinking of the protoplasm away from the intact cell wall) and cell death. This fact explains why brines and layering meat and fish in salt are time-honored methods of preserving food. Microorganisms called **halophiles** (“salt loving”) actually require high salt concentrations for growth. These organisms are found in marine environments where salt concentrations hover at 3.5%. Extreme halophilic microorganisms, such as the red alga

Dunaliella salina and the archaeal species *Halobacterium* in [\[link\]](#), grow in hypersaline lakes such as the Great Salt Lake, which is 3.5–8 times saltier than the ocean, and the Dead Sea, which is 10 times saltier than the ocean.



Photograph taken from space of the Great Salt Lake in Utah. The purple color is caused by high density of the alga *Dunaliella* and the archaean *Halobacterium* spp. (credit: NASA)

Dunaliella spp. counters the tremendous osmotic pressure of the environment with a high cytoplasmic concentration of glycerol and by actively pumping out salt ions. *Halobacterium* spp. accumulates large concentrations of K^+ and other ions in its cytoplasm. Its proteins are designed for high salt concentrations and lose activity at salt concentrations below 1–2 M. Although most **halotolerant** organisms, for example *Halomonas* spp. in salt marshes, do not need high concentrations of salt for

growth, they will survive and divide in the presence of high salt. Not surprisingly, the staphylococci, micrococci, and corynebacteria that colonize our skin tolerate salt in their environment. Halotolerant pathogens are an important cause of food-borne illnesses because they survive and multiply in salty food. For example, the halotolerant bacteria *S. aureus*, *Bacillus cereus*, and *V. cholerae* produce dangerous enterotoxins and are major causes of food poisoning.

Microorganisms depend on available water to grow. Available moisture is measured as water activity (a_w), which is the ratio of the vapor pressure of the medium of interest to the vapor pressure of pure distilled water; therefore, the a_w of water is equal to 1.0. Bacteria require high a_w (0.97–0.99), whereas fungi can tolerate drier environments; for example, the range of a_w for growth of *Aspergillus* spp. is 0.8–0.75. Decreasing the water content of foods by drying, as in jerky, or through freeze-drying or by increasing osmotic pressure, as in brine and jams, are common methods of preventing spoilage.

Microorganisms that require high atmospheric pressure for growth are called **barophiles**. The bacteria that live at the bottom of the ocean must be able to withstand great pressures. Because it is difficult to retrieve intact specimens and reproduce such growth conditions in the laboratory, the characteristics of these microorganisms are largely unknown.

Light

Photoautotrophs, such as cyanobacteria or green sulfur bacteria, and photoheterotrophs, such as purple nonsulfur bacteria, depend on sufficient light intensity at the wavelengths absorbed by their pigments to grow and multiply. Energy from light is captured by pigments and converted into chemical energy that drives carbon fixation and other metabolic processes. The portion of the electromagnetic spectrum that is absorbed by these organisms is defined as photosynthetically active radiation (PAR). It lies within the visible light spectrum ranging from 400 to 700 nanometers (nm) and extends in the near infrared for some photosynthetic bacteria. A number of accessory pigments, such as fucoxanthin in brown algae and phycobilins in cyanobacteria, widen the useful range of wavelengths for photosynthesis

and compensate for the low light levels available at greater depths of water. Other microorganisms, such as the archaea of the class Halobacteria, use light energy to drive their proton and sodium pumps. The light is absorbed by a pigment protein complex called bacteriorhodopsin, which is similar to the eye pigment rhodopsin. Photosynthetic bacteria are present not only in aquatic environments but also in soil and in symbiosis with fungi in lichens. The peculiar watermelon snow is caused by a microalga *Chlamydomonas nivalis*, a green alga rich in a secondary red carotenoid pigment (astaxanthin) which gives the pink hue to the snow where the alga grows.

Note:

- Which photosynthetic pigments were described in this section?
- What is the fundamental stress of a hypersaline environment for a cell?

Key Concepts and Summary

- **Halophiles** require high salt concentration in the medium, whereas **halotolerant** organisms can grow and multiply in the presence of high salt but do not require it for growth.
- Halotolerant pathogens are an important source of foodborne illnesses because they contaminate foods preserved in salt.
- Photosynthetic bacteria depend on visible light for energy.
- Most bacteria, with few exceptions, require high moisture to grow.

Multiple Choice

Exercise:

Problem:

Which of the following is the reason jams and dried meats often do not require refrigeration to prevent spoilage?

- A. low pH
- B. toxic alkaline chemicals
- C. naturally occurring antibiotics
- D. low water activity

Solution:

D

Exercise:**Problem:**

Bacteria living in salt marshes are most likely which of the following?

- A. acidophiles
- B. barophiles
- C. halotolerant
- D. thermophiles

Solution:

C

Fill in the Blank**Exercise:****Problem:**

A bacterium that thrives in the Great Salt Lake but not in fresh water is probably a _____.

Solution:

halophile

Exercise:**Problem:**

Bacteria isolated from the bottom of the ocean need high atmospheric pressures to survive. They are _____.

Solution:

barophiles

Exercise:**Problem:**

Staphylococcus aureus can be grown on multipurpose growth medium or on mannitol salt agar that contains 7.5% NaCl. The bacterium is _____.

Solution:

halotolerant

Short Answer**Exercise:****Problem:**

Fish sauce is a salty condiment produced using fermentation. What type of organism is likely responsible for the fermentation of the fish sauce?

Media Used for Bacterial Growth

LEARNING OBJECTIVES

- Identify and describe culture media for the growth of bacteria, including examples of all-purpose media, enriched, selective, differential, defined, and enrichment media

The study of microorganisms is greatly facilitated if we are able to culture them, that is, to keep reproducing populations alive under laboratory conditions. Culturing many microorganisms is challenging because of highly specific nutritional and environmental requirements and the diversity of these requirements among different species.

Nutritional Requirements

The number of available media to grow bacteria is considerable. Some media are considered general all-purpose media and support growth of a large variety of organisms. A prime example of an all-purpose medium is tryptic soy broth (TSB). Specialized media are used in the identification of bacteria and are supplemented with dyes, pH indicators, or antibiotics. One type, **enriched media**, contains growth factors, vitamins, and other essential nutrients to promote the growth of **fastidious organisms**, organisms that cannot make certain nutrients and require them to be added to the medium. When the complete chemical composition of a medium is known, it is called a **chemically defined medium**. For example, in EZ medium, all individual chemical components are identified and the exact amounts of each is known. In **complex media**, which contain extracts and digests of yeasts, meat, or plants, the precise chemical composition of the

medium is not known. Amounts of individual components are undetermined and variable. Nutrient broth, tryptic soy broth, and brain heart infusion, are all examples of complex media.

Media that inhibit the growth of unwanted microorganisms and support the growth of the organism of interest by supplying nutrients and reducing competition are called **selective media**. An example of a selective medium is MacConkey agar. It contains bile salts and crystal violet, which interfere with the growth of many gram-positive bacteria and favor the growth of gram-negative bacteria, particularly the Enterobacteriaceae. These species are commonly named enterics, reside in the intestine, and are adapted to the presence of bile salts. The **enrichment cultures** foster the preferential growth of a desired microorganism that represents a fraction of the organisms present in an inoculum. For example, if we want to isolate bacteria that break down crude oil, hydrocarbonoclastic bacteria, sequential subculturing in a medium that supplies carbon only in the form of crude oil will enrich the cultures with oil-eating bacteria. The **differential media** make it easy to distinguish colonies of different bacteria by a change in the color of the colonies or the color of the medium. Color changes are the result of end products created by interaction of bacterial enzymes with differential substrates in the medium or, in the case of hemolytic reactions, the lysis of red blood cells in the medium. In [\[link\]](#), the differential fermentation of lactose can be observed on MacConkey agar. The lactose fermenters produce acid, which turns the medium and the colonies of strong fermenters hot pink. The medium is supplemented with the pH indicator neutral red, which turns to hot pink at low pH. Selective and differential media can be combined and play an important role in the identification of bacteria by biochemical methods.



On this MacConkey agar plate, the lactose-fermenter *E. coli* colonies are bright pink. *Serratia marcescens*, which does not ferment lactose, forms a cream-colored streak on the tan medium. (credit: American Society for Microbiology)

Note:

- Distinguish complex and chemically defined media.
- Distinguish selective and enrichment media.

Note:

Compare the compositions of [EZ medium](#) and [sheep blood](#) agar.

Note:**The End-of-Year Picnic**

The microbiology department is celebrating the end of the school year in May by holding its traditional picnic on the green. The speeches drag on for a couple of hours, but finally all the faculty and students can dig into the food: chicken salad, tomatoes, onions, salad, and custard pie. By evening, the whole department, except for two vegetarian students who did not eat the chicken salad, is stricken with nausea, vomiting, retching, and abdominal cramping. Several individuals complain of diarrhea. One patient shows signs of shock (low blood pressure). Blood and stool samples are collected from patients, and an analysis of all foods served at the meal is conducted.

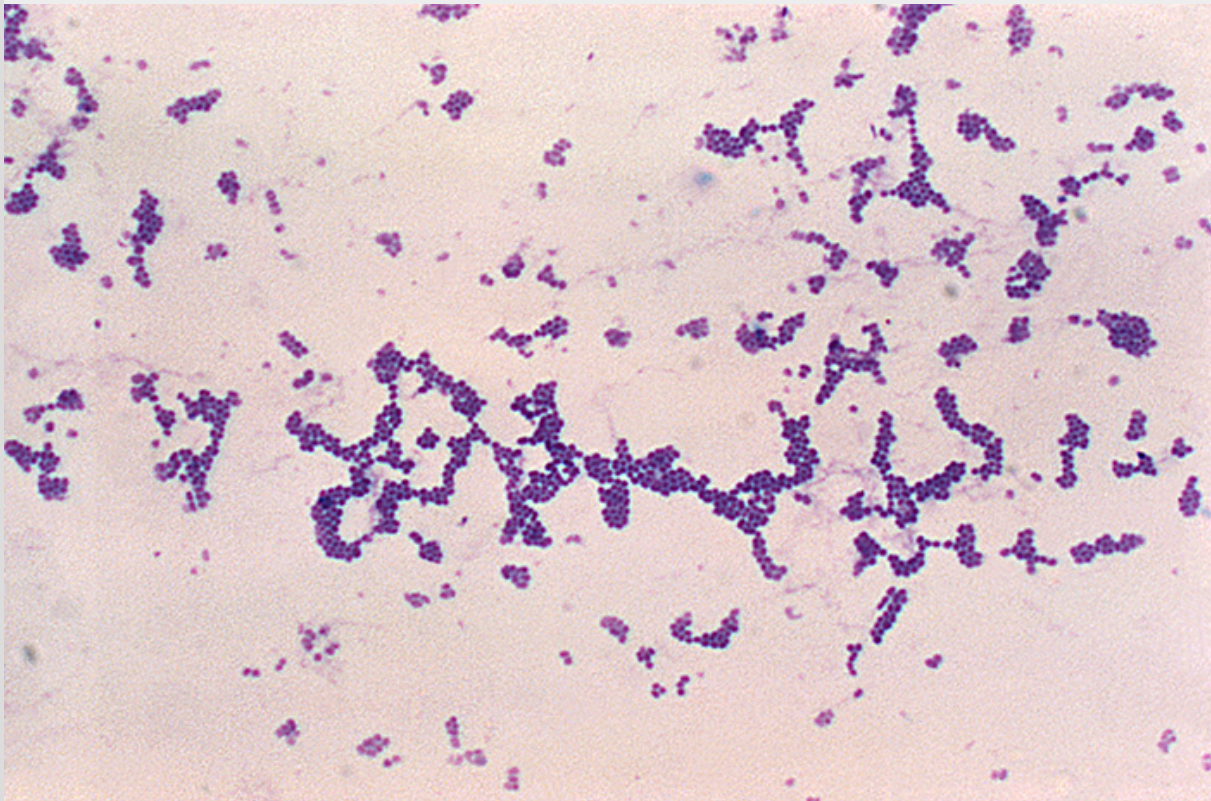
Bacteria can cause gastroenteritis (inflammation of the stomach and intestinal tract) either by colonizing and replicating in the host, which is considered an infection, or by secreting toxins, which is considered intoxication. Signs and symptoms of infections are typically delayed, whereas intoxication manifests within hours, as happened after the picnic. Blood samples from the patients showed no signs of bacterial infection, which further suggests that this was a case of intoxication. Since intoxication is due to secreted toxins, bacteria are not usually detected in blood or stool samples. MacConkey agar and sorbitol-MacConkey agar plates and xylose-lysine-deoxycholate (XLD) plates were inoculated with stool samples and did not reveal any unusually colored colonies, and no black colonies or white colonies were observed on XLD. All lactose fermenters on MacConkey agar also ferment sorbitol. These results ruled out common agents of food-borne illnesses: *E. coli*, *Salmonella* spp., and *Shigella* spp.

Analysis of the chicken salad revealed an abnormal number of gram-positive cocci arranged in clusters ([\[link\]](#)). A culture of the gram-positive cocci releases bubbles when mixed with hydrogen peroxide. The culture turned mannitol salt agar yellow after a 24-hour incubation.

All the tests point to *Staphylococcus aureus* as the organism that secreted the toxin. Samples from the salad showed the presence of gram-positive cocci bacteria in clusters. The colonies were positive for catalase. The bacteria grew on mannitol salt agar fermenting mannitol, as shown by the change to yellow of the medium. The pH indicator in mannitol salt agar is phenol red, which turns to yellow when the medium is acidified by the products of fermentation.

The toxin secreted by *S. aureus* is known to cause severe gastroenteritis. The organism was probably introduced into the salad during preparation by the food handler and multiplied while the salad was kept in the warm ambient temperature during the speeches.

- What are some other factors that might have contributed to rapid growth of *S. aureus* in the chicken salad?
- Why would *S. aureus* not be inhibited by the presence of salt in the chicken salad?



Gram-positive cocci in clusters. (credit: Centers for Disease Control and Prevention)

Key Concepts and Summary

- **Chemically defined media** contain only chemically known components.
- **Selective media** favor the growth of some microorganisms while inhibiting others.
- **Enriched media** contain added essential nutrients a specific organism needs to grow
- **Differential media** help distinguish bacteria by the color of the colonies or the change in the medium.

Multiple Choice

Exercise:

Problem:

EMB agar is a medium used in the identification and isolation of pathogenic bacteria. It contains digested meat proteins as a source of organic nutrients. Two indicator dyes, eosin and methylene blue, inhibit the growth of gram-positive bacteria and distinguish between lactose fermenting and nonlactose fermenting organisms. Lactose fermenters form metallic green or deep purple colonies, whereas the nonlactose fermenters form completely colorless colonies. EMB agar is an example of which of the following?

- A. a selective medium only
- B. a differential medium only
- C. a selective medium and a chemically defined medium
- D. a selective medium, a differential medium, and a complex medium

Solution:

D

Exercise:**Problem:**

Haemophilus influenzae must be grown on chocolate agar, which is blood agar treated with heat to release growth factors in the medium. *H. influenzae* is described as _____.

- A. an acidophile
- B. a thermophile
- C. an obligate anaerobe
- D. fastidious

Solution:

D

Fill in the Blank**Exercise:****Problem:**

Blood agar contains many unspecified nutrients, supports the growth of a large number of bacteria, and allows differentiation of bacteria according to hemolysis (breakdown of blood). The medium is _____ and _____.

Solution:

complex, differential

Exercise:

Problem:

Rogosa agar contains yeast extract. The pH is adjusted to 5.2 and discourages the growth of many microorganisms; however, all the colonies look similar. The medium is _____ and _____.

Solution:

complex, selective

Short Answer**Exercise:****Problem:**

What is the major difference between an enrichment culture and a selective culture?

Critical Thinking**Exercise:****Problem:**

Haemophilus influenzae grows best at 35–37 °C with ~5% CO₂ (or in a candle-jar) and requires hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth. (Centers for Disease Control and Prevention, World Health Organization. “*CDC Laboratory Methods for the Diagnosis of Meningitis Caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae*. WHO Manual, 2nd edition.” 2011.

<http://www.cdc.gov/meningitis/lab-manual/full-manual.pdf>) Using the vocabulary learned in this chapter, describe *H. influenzae*.

Introduction

class="introduction"

Most environments, including cars, are not sterile.

A study[[footnote](#)] analyzed 11 locations within 18 different cars to determine the number of microbial colony-forming units (CFUs) present. The center console harbored by far the most microbes (506 CFUs), possibly because that is where drinks are placed (and often spilled). Frequently touched sites also had high concentrations. (credit “photo”: modification

of work by Jeff
 Wilcox)
 R.E.
 Stephenson et
 al.
 “Elucidation of
 Bacteria Found
 in Car Interiors
 and Strategies
 to Reduce the
 Presence of
 Potential
 Pathogens.”
Biofouling 30
 no. 3
 (2014):337–
 346.

Location	Average number CFUs per 6.5 × 6.5 cm area
Door latch	256
Door lock	14
Door lock control	182
Door handle	29
Window control	4
Cruise control button	69
Steering wheel	239
Interior steering wheel	390
Radio volume knob	99
Gear shifter	115
Center console	506



How clean is clean? People wash their cars and vacuum the carpets, but most would not want to eat from these surfaces. Similarly, we might eat with silverware cleaned in a dishwasher, but we could not use the same dishwasher to clean surgical instruments. As these examples illustrate, “clean” is a relative term. Car washing, vacuuming, and dishwashing all reduce the microbial load on the items treated, thus making them “cleaner.” But whether they are “clean enough” depends on their intended use.

Because people do not normally eat from cars or carpets, these items do not require the same level of cleanliness that silverware does. Likewise, because silverware is not used for invasive surgery, these utensils do not require the same level of cleanliness as surgical equipment, which requires sterilization to prevent infection.

Why not play it safe and sterilize everything? Sterilizing everything we come in contact with is impractical, as well as potentially dangerous. As this chapter will demonstrate, sterilization protocols often require time- and labor-intensive treatments that may degrade the quality of the item being treated or have toxic effects on users. Therefore, the user must consider the item's intended application when choosing a cleaning method to ensure that it is "clean enough."

Controlling Microbial Growth

LEARNING OBJECTIVES

- Compare disinfectants, antiseptics, and sterilants
- Describe the principles of controlling the presence of microorganisms through sterilization and disinfection
- Differentiate between microorganisms of various biological safety levels and explain methods used for handling microbes at each level

Note:

Part 1

Roberta is a 46-year-old real estate agent who recently underwent a cholecystectomy (surgery to remove painful gallstones). The surgery was performed laparoscopically with the aid of a duodenoscope, a specialized endoscope that allows surgeons to see inside the body with the aid of a tiny camera. On returning home from the hospital, Roberta developed abdominal pain and a high fever. She also experienced a burning sensation during urination and noticed blood in her urine. She notified her surgeon of these symptoms, per her postoperative instructions.

- What are some possible causes of Roberta's symptoms?

Jump to the [next](#) Clinical Focus box.

To prevent the spread of human disease, it is necessary to control the growth and abundance of microbes in or on various items frequently used by humans. Inanimate items, such as doorknobs, toys, or towels, which may harbor microbes and aid in disease transmission, are called **fomites**. Two factors heavily influence the level of cleanliness required for a particular fomite and, hence, the protocol chosen to achieve this level. The first factor is the application for which the item will be used. For example, invasive applications that require insertion into the human body require a much higher level of cleanliness than applications that do not. The second factor is the level of resistance to antimicrobial treatment by potential pathogens. For example, foods preserved by canning often become contaminated with the bacterium *Clostridium botulinum*, which produces the neurotoxin that causes botulism. Because *C. botulinum* can produce endospores that can survive harsh conditions, extreme temperatures and pressures must be used to eliminate the endospores. Other organisms may not require such extreme measures and can be controlled by a procedure such as washing clothes in a laundry machine.

Laboratory Biological Safety Levels

For researchers or laboratory personnel working with pathogens, the risks associated with specific pathogens determine the levels of cleanliness and control required. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have established four classification levels, called “biological safety levels” (BSLs). Various organizations around the world, including the World Health Organization (WHO) and the European Union (EU), use a similar classification scheme. According to the CDC, the BSL is determined by the agent’s infectivity, ease of transmission, and potential disease severity, as well as the type of work being done with the agent.[\[footnote\]](#)

US Centers for Disease Control and Prevention. “Recognizing the Biosafety Levels.” <http://www.cdc.gov/training/quicklearns/biosafety/>. Accessed June 7, 2016.

Each BSL requires a different level of biocontainment to prevent contamination and spread of infectious agents to laboratory personnel and, ultimately, the community. For example, the lowest BSL, BSL-1, requires

the fewest precautions because it applies to situations with the lowest risk for microbial infection.

BSL-1 agents are those that generally do not cause infection in healthy human adults. These include noninfectious bacteria, such as nonpathogenic strains of *Escherichia coli* and *Bacillus subtilis*, and viruses known to infect animals other than humans, such as baculoviruses (insect viruses). Because working with BSL-1 agents poses very little risk, few precautions are necessary. Laboratory workers use standard aseptic technique and may work with these agents at an open laboratory bench or table, wearing personal protective equipment (PPE) such as a laboratory coat, goggles, and gloves, as needed. Other than a sink for handwashing and doors to separate the laboratory from the rest of the building, no additional modifications are needed.

Agents classified as BSL-2 include those that pose moderate risk to laboratory workers and the community, and are typically “indigenous,” meaning that they are commonly found in that geographical area. These include bacteria such as *Staphylococcus aureus* and *Salmonella* spp., and viruses like hepatitis, mumps, and measles viruses. BSL-2 laboratories require additional precautions beyond those of BSL-1, including restricted access; required PPE, including a face shield in some circumstances; and the use of biological safety cabinets for procedures that may disperse agents through the air (called “aerosolization”). BSL-2 laboratories are equipped with self-closing doors, an eyewash station, and an **autoclave**, which is a specialized device for sterilizing materials with pressurized steam before use or disposal. BSL-1 laboratories may also have an autoclave.

BSL-3 agents have the potential to cause lethal infections by inhalation. These may be either indigenous or “exotic,” meaning that they are derived from a foreign location, and include pathogens such as *Mycobacterium tuberculosis*, *Bacillus anthracis*, West Nile virus, and human immunodeficiency virus (HIV). Because of the serious nature of the infections caused by BSL-3 agents, laboratories working with them require restricted access. Laboratory workers are under medical surveillance, possibly receiving vaccinations for the microbes with which they work. In addition to the standard PPE already mentioned, laboratory personnel in

BSL-3 laboratories must also wear a respirator and work with microbes and infectious agents in a biological safety cabinet at all times. BSL-3 laboratories require a hands-free sink, an eyewash station near the exit, and two sets of self-closing and locking doors at the entrance. These laboratories are equipped with directional airflow, meaning that clean air is pulled through the laboratory from clean areas to potentially contaminated areas. This air cannot be recirculated, so a constant supply of clean air is required.

BSL-4 agents are the most dangerous and often fatal. These microbes are typically exotic, are easily transmitted by inhalation, and cause infections for which there are no treatments or vaccinations. Examples include Ebola virus and Marburg virus, both of which cause hemorrhagic fevers, and smallpox virus. There are only a small number of laboratories in the United States and around the world appropriately equipped to work with these agents. In addition to BSL-3 precautions, laboratory workers in BSL-4 facilities must also change their clothing on entering the laboratory, shower on exiting, and decontaminate all material on exiting. While working in the laboratory, they must either wear a full-body protective suit with a designated air supply or conduct all work within a biological safety cabinet with a high-efficiency particulate air (HEPA)-filtered air supply and a doubly HEPA-filtered exhaust. If wearing a suit, the air pressure within the suit must be higher than that outside the suit, so that if a leak in the suit occurs, laboratory air that may be contaminated cannot be drawn into the suit ([\[link\]](#)). The laboratory itself must be located either in a separate building or in an isolated portion of a building and have its own air supply and exhaust system, as well as its own decontamination system. The BSLs are summarized in [\[link\]](#).



A protective suit like this one is an additional precaution for those who work in BSL-4 laboratories. This suit has its own air supply and maintains a positive pressure relative to the outside, so that if a leak occurs, air will flow out of the suit, not into it from the laboratory. (credit: modification of work by Centers for Disease Control and Prevention)

Biosafety Levels			
Biological Safety Levels	Description	Examples	CDC Classification
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	<i>Mycobacterium tuberculosis</i>	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	<i>Staphylococcus aureus</i>	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of <i>Escherichia coli</i>	

The CDC classifies infectious agents into four biosafety levels based on potential risk to laboratory personnel and the community. Each level requires a progressively greater level of precaution. (credit “pyramid”: modification of work by Centers for Disease Control and Prevention)

Note:

To [learn more](#) about the four BSLs, visit the CDC’s website.

Note:

- What are some factors used to determine the BSL necessary for working with a specific pathogen?

Sterilization

The most extreme protocols for microbial control aim to achieve **sterilization**: the complete removal or killing of all vegetative cells, endospores, and viruses from the targeted item or environment. Sterilization protocols are generally reserved for laboratory, medical, manufacturing, and food industry settings, where it may be imperative for certain items to be completely free of potentially infectious agents. Sterilization can be accomplished through either physical means, such as exposure to high heat, pressure, or filtration through an appropriate filter, or by chemical means. Chemicals that can be used to achieve sterilization are called **sterilants**. Sterilants effectively kill all microbes and viruses, and, with appropriate exposure time, can also kill endospores.

For many clinical purposes, **aseptic technique** is necessary to prevent contamination of sterile surfaces. Aseptic technique involves a combination of protocols that collectively maintain sterility, or **asepsis**, thus preventing contamination of the patient with microbes and infectious agents. Failure to practice aseptic technique during many types of clinical procedures may introduce microbes to the patient's body and put the patient at risk for **sepsis**, a systemic inflammatory response to an infection that results in high fever, increased heart and respiratory rates, shock, and, possibly, death. Medical procedures that carry risk of contamination must be performed in a **sterile field**, a designated area that is kept free of all vegetative microbes, endospores, and viruses. Sterile fields are created according to protocols requiring the use of sterilized materials, such as packaging and drapings, and strict procedures for washing and application of sterilants. Other protocols are followed to maintain the sterile field while the medical procedure is being performed.

One food sterilization protocol, **commercial sterilization**, uses heat at a temperature low enough to preserve food quality but high enough to destroy common pathogens responsible for food poisoning, such as *C. botulinum*. Because *C. botulinum* and its endospores are commonly found in soil, they

may easily contaminate crops during harvesting, and these endospores can later germinate within the anaerobic environment once foods are canned. Metal cans of food contaminated with *C. botulinum* will bulge due to the microbe's production of gases; contaminated jars of food typically bulge at the metal lid. To eliminate the risk for *C. botulinum* contamination, commercial food-canning protocols are designed with a large margin of error. They assume an impossibly large population of endospores (10^{12} per can) and aim to reduce this population to 1 endospore per can to ensure the safety of canned foods. For example, low- and medium-acid foods are heated to 121 °C for a minimum of 2.52 minutes, which is the time it would take to reduce a population of 10^{12} endospores per can down to 1 endospore at this temperature. Even so, commercial sterilization does not eliminate the presence of all microbes; rather, it targets those pathogens that cause spoilage and foodborne diseases, while allowing many nonpathogenic organisms to survive. Therefore, “sterilization” is somewhat of a misnomer in this context, and commercial sterilization may be more accurately described as “quasi-sterilization.”

Note:

- What is the difference between sterilization and aseptic technique?

Note:

The Association of Surgical Technologists publishes [standards](#) for aseptic technique, including creating and maintaining a sterile field.

Other Methods of Control

Sterilization protocols require procedures that are not practical, or necessary, in many settings. Various other methods are used in clinical and nonclinical settings to reduce the microbial load on items. Although the

terms for these methods are often used interchangeably, there are important distinctions ([link](#)).

The process of **disinfection** inactivates most microbes on the surface of a fomite by using antimicrobial chemicals or heat. Because some microbes remain, the disinfected item is not considered sterile. Ideally, **disinfectants** should be fast acting, stable, easy to prepare, inexpensive, and easy to use. An example of a natural disinfectant is vinegar; its acidity kills most microbes. Chemical disinfectants, such as chlorine bleach or products containing chlorine, are used to clean nonliving surfaces such as laboratory benches, clinical surfaces, and bathroom sinks. Typical disinfection does not lead to sterilization because endospores tend to survive even when all vegetative cells have been killed.

Unlike disinfectants, **antiseptics** are antimicrobial chemicals safe for use on living skin or tissues. Examples of antiseptics include hydrogen peroxide and isopropyl alcohol. The process of applying an antiseptic is called **antiseptis**. In addition to the characteristics of a good disinfectant, antiseptics must also be selectively effective against microorganisms and able to penetrate tissue deeply without causing tissue damage.

The type of protocol required to achieve the desired level of cleanliness depends on the particular item to be cleaned. For example, those used clinically are categorized as critical, semicritical, and noncritical. Critical items must be sterile because they will be used inside the body, often penetrating sterile tissues or the bloodstream; examples of **critical items** include surgical instruments, catheters, and intravenous fluids. Gastrointestinal endoscopes and various types of equipment for respiratory therapies are examples of **semicritical items**; they may contact mucous membranes or nonintact skin but do not penetrate tissues. Semicritical items do not typically need to be sterilized but do require a high level of disinfection. Items that may contact but not penetrate intact skin are **noncritical items**; examples are bed linens, furniture, crutches, stethoscopes, and blood pressure cuffs. These articles need to be clean but not highly disinfected.

The act of handwashing is an example of **degerming**, in which microbial numbers are significantly reduced by gently scrubbing living tissue, most

commonly skin, with a mild chemical (e.g., soap) to avoid the transmission of pathogenic microbes. Wiping the skin with an alcohol swab at an injection site is another example of degerming. These degerming methods remove most (but not all) microbes from the skin's surface.

The term **sanitization** refers to the cleansing of fomites to remove enough microbes to achieve levels deemed safe for public health. For example, commercial dishwashers used in the food service industry typically use very hot water and air for washing and drying; the high temperatures kill most microbes, sanitizing the dishes. Surfaces in hospital rooms are commonly sanitized using a chemical disinfectant to prevent disease transmission between patients. [\[link\]](#) summarizes common protocols, definitions, applications, and agents used to control microbial growth.

Common Protocols for Control of Microbial Growth			
Protocol	Definition	Common Application	Common Agents
For Use on Fomites			
Disinfection	Reduces or destroys microbial load of an inanimate item through application of heat or antimicrobial chemicals	Cleaning surfaces like laboratory benches, clinical surfaces, and bathrooms	Chlorine bleach, phenols (e.g., Lysol), glutaraldehyde
Sanitization	Reduces microbial load of an inanimate item to safe public health levels through application of heat or antimicrobial chemicals	Commercial dishwashing of eating utensils, cleaning public restrooms	Detergents containing phosphates (e.g., Finish), industrial-strength cleaners containing quaternary ammonium compounds
Sterilization	Completely eliminates all vegetative cells, endospores, and viruses from an inanimate item	Preparation of surgical equipment and of needles used for injection	Pressurized steam (autoclave), chemicals, radiation
For Use on Living Tissue			
Antisepsis	Reduces microbial load on skin or tissue through application of an antimicrobial chemical	Cleaning skin broken due to injury; cleaning skin before surgery	Boric acid, isopropyl alcohol, hydrogen peroxide, iodine (betadine)
Degerming	Reduces microbial load on skin or tissue through gentle to firm scrubbing and the use of mild chemicals	Handwashing	Soap, alcohol swab

Note:

- What is the difference between a disinfectant and an antiseptic?
- Which is most effective at removing microbes from a product: sanitization, degerming, or sterilization? Explain.

Note:

Part 2

Roberta's physician suspected that a bacterial infection was responsible for her sudden-onset high fever, abdominal pain, and bloody urine. Based on these symptoms, the physician diagnosed a urinary tract infection (UTI). A wide variety of bacteria may cause UTIs, which typically occur when bacteria from the lower gastrointestinal tract are introduced to the urinary tract. However, Roberta's recent gallstone surgery caused the physician to suspect that she had contracted a nosocomial (hospital-acquired) infection during her surgery. The physician took a urine sample and ordered a urine culture to check for the presence of white blood cells, red blood cells, and bacteria. The results of this test would help determine the cause of the infection. The physician also prescribed a course of the antibiotic ciprofloxacin, confident that it would clear Roberta's infection.

- What are some possible ways that bacteria could have been introduced to Roberta's urinary tract during her surgery?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

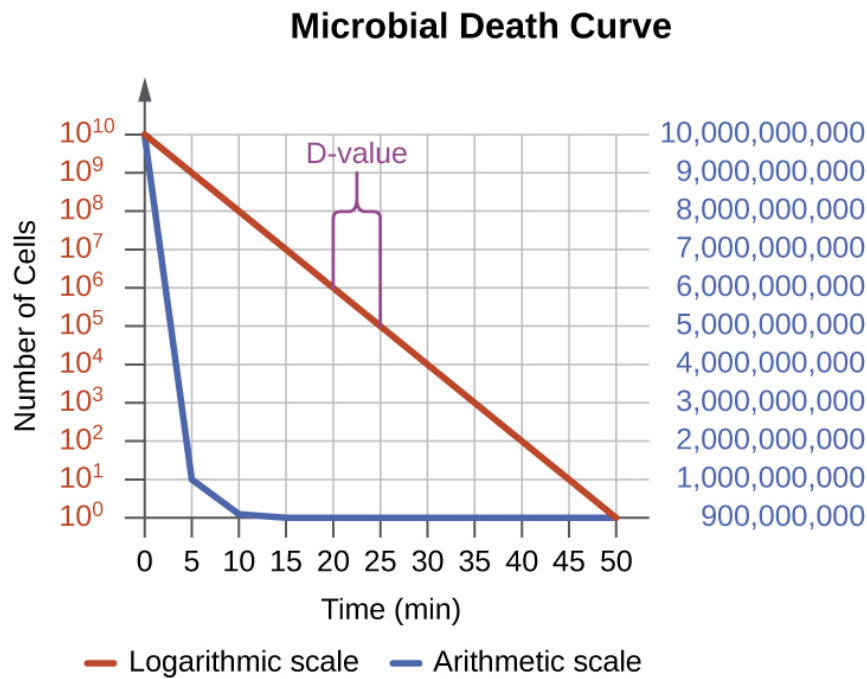
Measuring Microbial Control

Physical and chemical methods of microbial control that kill the targeted microorganism are identified by the suffix *-cide* (or *-cidal*). The prefix indicates the type of microbe or infectious agent killed by the treatment method: **bactericides** kill bacteria, **viricides** kill or inactivate viruses, and

fungicides kill fungi. Other methods do not kill organisms but, instead, stop their growth, making their population static; such methods are identified by the suffix *-stat* (or *-static*). For example, **bacteriostatic** treatments inhibit the growth of bacteria, whereas **fungistatic** treatments inhibit the growth of fungi. Factors that determine whether a particular treatment is *-cidal* or *-static* include the types of microorganisms targeted, the concentration of the chemical used, and the nature of the treatment applied.

Although *-static* treatments do not actually kill infectious agents, they are often less toxic to humans and other animals, and may also better preserve the integrity of the item treated. Such treatments are typically sufficient to keep the microbial population of an item in check. The reduced toxicity of some of these *-static* chemicals also allows them to be impregnated safely into plastics to prevent the growth of microbes on these surfaces. Such plastics are used in products such as toys for children and cutting boards for food preparation. When used to treat an infection, *-static* treatments are typically sufficient in an otherwise healthy individual, preventing the pathogen from multiplying, thus allowing the individual's immune system to clear the infection.

The degree of microbial control can be evaluated using a **microbial death curve** to describe the progress and effectiveness of a particular protocol. When exposed to a particular microbial control protocol, a fixed percentage of the microbes within the population will die. Because the rate of killing remains constant even when the population size varies, the percentage killed is more useful information than the absolute number of microbes killed. Death curves are often plotted as semilog plots just like microbial growth curves because the reduction in microorganisms is typically logarithmic ([\[link\]](#)). The amount of time it takes for a specific protocol to produce a one order-of-magnitude decrease in the number of organisms, or the death of 90% of the population, is called the **decimal reduction time (DRT)** or **D-value**.



Time (min)	Number of cells
0	10^{10}
5	10^9
10	10^8
15	10^7
20	10^6
25	10^5
30	10^4
35	10^3
40	10^2
45	10^1
50	10^0

Microbial death is logarithmic and easily observed using a semilog plot instead of an arithmetic one. The decimal reduction time (D-value) is the time it takes to kill 90% of the population (a 1-log decrease in the total population) when exposed to a specific microbial control protocol, as indicated by the purple bracket.

Several factors contribute to the effectiveness of a disinfecting agent or microbial control protocol. First, as demonstrated in [\[link\]](#), the length of time of exposure is important. Longer exposure times kill more microbes. Because microbial death of a population exposed to a specific protocol is logarithmic, it takes longer to kill a high-population load than a low-population load exposed to the same protocol. A shorter treatment time (measured in multiples of the D-value) is needed when starting with a smaller number of organisms. Effectiveness also depends on the susceptibility of the agent to that disinfecting agent or protocol. The concentration of disinfecting agent or intensity of exposure is also important. For example, higher temperatures and higher concentrations of

disinfectants kill microbes more quickly and effectively. Conditions that limit contact between the agent and the targeted cells—for example, the presence of bodily fluids, tissue, organic debris (e.g., mud or feces), or biofilms on surfaces—increase the cleaning time or intensity of the microbial control protocol required to reach the desired level of cleanliness. All these factors must be considered when choosing the appropriate protocol to control microbial growth in a given situation.

Note:

- What are two possible reasons for choosing a bacteriostatic treatment over a bactericidal one?
- Name at least two factors that can compromise the effectiveness of a disinfecting agent.

Key Concepts and Summary

- Inanimate items that may harbor microbes and aid in their transmission are called **fomites**. The level of cleanliness required for a fomite depends both on the item's use and the infectious agent with which the item may be contaminated.
- The CDC and the NIH have established four **biological safety levels (BSLs)** for laboratories performing research on infectious agents. Each level is designed to protect laboratory personnel and the community. These BSLs are determined by the agent's infectivity, ease of transmission, and potential disease severity, as well as the type of work being performed with the agent.
- **Disinfection** removes potential pathogens from a fomite, whereas **antisepsis** uses antimicrobial chemicals safe enough for tissues; in both cases, microbial load is reduced, but microbes may remain unless the chemical used is strong enough to be a **sterilant**.
- The amount of cleanliness (**sterilization** versus high-level disinfection versus general cleanliness) required for items used clinically depends

on whether the item will come into contact with sterile tissues (**critical item**), mucous membranes (**semicritical item**), or intact skin (**noncritical item**).

- Medical procedures with a risk for contamination should be carried out in a **sterile field** maintained by proper **aseptic technique** to prevent **sepsis**.
- Sterilization is necessary for some medical applications as well as in the food industry, where endospores of *Clostridium botulinum* are killed through **commercial sterilization** protocols.
- Physical or chemical methods to control microbial growth that result in death of the microbe are indicated by the suffixes *-cide* or *-cidal* (e.g., as with **bactericides**, **viricides**, and **fungicides**), whereas those that inhibit microbial growth are indicated by the suffixes *-stat* or *-static* (e.g., **bacteriostatic**, **fungistatic**).
- **Microbial death curves** display the logarithmic decline of living microbes exposed to a method of microbial control. The time it takes for a protocol to yield a 1-log (90%) reduction in the microbial population is the **decimal reduction time**, or **D-value**.
- When choosing a microbial control protocol, factors to consider include the length of exposure time, the type of microbe targeted, its susceptibility to the protocol, the intensity of the treatment, the presence of organics that may interfere with the protocol, and the environmental conditions that may alter the effectiveness of the protocol.

Multiple Choice

Exercise:

Problem:

Which of the following types of medical items requires sterilization?

- A. needles
- B. bed linens
- C. respiratory masks
- D. blood pressure cuffs

Solution:

A

Exercise:

Problem:

Which of the following is suitable for use on tissues for microbial control to prevent infection?

- A. disinfectant
- B. antiseptic
- C. sterilant
- D. water

Solution:

B

Exercise:

Problem:

Which biosafety level is appropriate for research with microbes or infectious agents that pose moderate risk to laboratory workers and the community, and are typically indigenous?

- A. BSL-1
- B. BSL-2
- C. BSL-3
- D. BSL-4

Solution:

B

Exercise:

Problem:

Which of the following best describes a microbial control protocol that inhibits the growth of molds and yeast?

- A. bacteriostatic
- B. fungicidal
- C. bactericidal
- D. fungistatic

Solution:

D

Exercise:**Problem:**

The decimal reduction time refers to the amount of time it takes to which of the following?

- A. reduce a microbial population by 10%
- B. reduce a microbial population by 0.1%
- C. reduce a microbial population by 90%
- D. completely eliminate a microbial population

Solution:

C

Fill in the Blank**Exercise:**

Problem:

A medical item that comes into contact with intact skin and does not penetrate sterile tissues or come into contact with mucous membranes is called a(n) _____ item.

Solution:

noncritical

Exercise:**Problem:**

The goal of _____ protocols is to rid canned produce of *Clostridium botulinum* endospores.

Solution:

commercial sterilization

True/False**Exercise:**

Problem: Sanitization leaves an object free of microbes.

Solution:

False

Short Answer**Exercise:**

Problem:

What are some characteristics of microbes and infectious agents that would require handling in a BSL-3 laboratory?

Exercise:

Problem:

What is the purpose of degerming? Does it completely eliminate microbes?

Exercise:

Problem:

What are some factors that alter the effectiveness of a disinfectant?

Critical Thinking

Exercise:

Problem:

When plotting microbial death curves, how might they look different for bactericidal versus bacteriostatic treatments?

Exercise:

Problem:

What are the benefits of cleaning something to a level of cleanliness beyond what is required? What are some possible disadvantages of doing so?

Introduction

class="introduction"

Although medical professionals rely heavily on signs and symptoms to diagnose disease and prescribe treatment, many diseases can produce similar signs and symptoms. (credit left: modification of work by U.S. Navy)



Jane woke up one spring morning feeling not quite herself. Her throat felt a bit dry and she was sniffing. She wondered why she felt so lousy. Was it because of a change in the weather? The pollen count? Was she coming down with something? Did she catch a bug from her coworker who sneezed on her in the elevator yesterday?

The signs and symptoms we associate with illness can have many different causes. Sometimes they are the direct result of a pathogenic infection, but in other cases they result from a response by our immune system to a pathogen or another perceived threat. For example, in response to certain pathogens, the immune system may release pyrogens, chemicals that cause the body temperature to rise, resulting in a fever. This response creates a less-than-favorable environment for the pathogen, but it also makes us feel sick.

Medical professionals rely heavily on analysis of signs and symptoms to determine the cause of an ailment and prescribe treatment. In some cases, signs and symptoms alone are enough to correctly identify the causative agent of a disease, but since few diseases produce truly unique symptoms, it is often necessary to confirm the identity of the infectious agent by other direct and indirect diagnostic methods.

Characteristics of Infectious Disease

LEARNING OBJECTIVES

- Distinguish between signs and symptoms of disease
- Explain the difference between a communicable disease and a noncommunicable disease
- Compare different types of infectious diseases, including iatrogenic, nosocomial, and zoonotic diseases
- Identify and describe the stages of an acute infectious disease in terms of number of pathogens present and severity of signs and symptoms

Note:

Part 1

Michael, a 10-year-old boy in generally good health, went to a birthday party on Sunday with his family. He ate many different foods but was the only one in the family to eat the undercooked hot dogs served by the hosts. Monday morning, he woke up feeling achy and nauseous, and he was running a fever of 38 °C (100.4 °F). His parents, assuming Michael had caught the flu, made him stay home from school and limited his activities. But after 4 days, Michael began to experience severe headaches, and his fever spiked to 40 °C (104 °F). Growing worried, his parents finally decide to take Michael to a nearby clinic.

- What signs and symptoms is Michael experiencing?
- What do these signs and symptoms tell us about the stage of Michael's disease?

Jump to the [next](#) Clinical Focus box.

A **disease** is any condition in which the normal structure or functions of the body are damaged or impaired. Physical injuries or disabilities are not classified as disease, but there can be several causes for disease, including infection by a pathogen, genetics (as in many cancers or deficiencies), noninfectious environmental causes, or inappropriate immune responses. Our focus in this chapter will be on infectious diseases, although when diagnosing infectious diseases, it is always important to consider possible noninfectious causes.

Signs and Symptoms of Disease

An **infection** is the successful colonization of a host by a microorganism. Infections can lead to disease, which causes signs and symptoms resulting in a deviation from the normal structure or functioning of the host. Microorganisms that can cause disease are known as pathogens.

The **signs** of disease are objective and measurable, and can be directly observed by a clinician. Vital signs, which are used to measure the body's basic functions, include body temperature (normally 37 °C [98.6 °F]), heart rate (normally 60–100 beats per minute), breathing rate (normally 12–18 breaths per minute), and blood pressure (normally between 90/60 and 120/80 mm Hg). Changes in any of the body's vital signs may be indicative of disease. For example, having a fever (a body temperature significantly higher than 37 °C or 98.6 °F) is a sign of disease because it can be measured.

In addition to changes in vital signs, other observable conditions may be considered signs of disease. For example, the presence of antibodies in a patient's serum (the liquid portion of blood that lacks clotting factors) can be observed and measured through blood tests and, therefore, can be considered a sign. However, it is important to note that the presence of antibodies is not always a sign of an active disease. Antibodies can remain

in the body long after an infection has resolved; also, they may develop in response to a pathogen that is in the body but not currently causing disease.

Unlike signs, **symptoms** of disease are subjective. Symptoms are felt or experienced by the patient, but they cannot be clinically confirmed or objectively measured. Examples of symptoms include nausea, loss of appetite, and pain. Such symptoms are important to consider when diagnosing disease, but they are subject to memory bias and are difficult to measure precisely. Some clinicians attempt to quantify symptoms by asking patients to assign a numerical value to their symptoms. For example, the Wong-Baker Faces pain-rating scale asks patients to rate their pain on a scale of 0–10. An alternative method of quantifying pain is measuring skin conductance fluctuations. These fluctuations reflect sweating due to skin sympathetic nerve activity resulting from the stressor of pain.[\[footnote\]](#)
F. Savino et al. “Pain Assessment in Children Undergoing Venipuncture: The Wong–Baker Faces Scale Versus Skin Conductance Fluctuations.” *PeerJ* 1 (2013):e37; <https://peerj.com/articles/37/>

A specific group of signs and symptoms characteristic of a particular disease is called a **syndrome**. Many syndromes are named using a nomenclature based on signs and symptoms or the location of the disease. [\[link\]](#) lists some of the prefixes and suffixes commonly used in naming syndromes.

Nomenclature of Symptoms		
Affix	Meaning	Example
cyto-	cell	cytopenia: reduction in the number of blood cells
hepat-	of the liver	hepatitis: inflammation of the liver

Nomenclature of Symptoms		
Affix	Meaning	Example
-pathy	disease	neuropathy: a disease affecting nerves
-emia	of the blood	bacteremia: presence of bacteria in blood
-itis	inflammation	colitis: inflammation of the colon
-lysis	destruction	hemolysis: destruction of red blood cells
-oma	tumor	lymphoma: cancer of the lymphatic system
-osis	diseased or abnormal condition	leukocytosis: abnormally high number of white blood cells
-derma	of the skin	keratoderma: a thickening of the skin

Clinicians must rely on signs and on asking questions about symptoms, medical history, and the patient's recent activities to identify a particular disease and the potential causative agent. Diagnosis is complicated by the fact that different microorganisms can cause similar signs and symptoms in a patient. For example, an individual presenting with symptoms of diarrhea may have been infected by one of a wide variety of pathogenic microorganisms. Bacterial pathogens associated with diarrheal disease include *Vibrio cholerae*, *Listeria monocytogenes*, *Campylobacter jejuni*, and enteropathogenic *Escherichia coli* (EPEC). Viral pathogens associated with diarrheal disease include norovirus and rotavirus. Parasitic pathogens associated with diarrhea include *Giardia lamblia* and *Cryptosporidium*

parvum. Likewise, fever is indicative of many types of infection, from the common cold to the deadly Ebola hemorrhagic fever.

Finally, some diseases may be **asymptomatic** or **subclinical**, meaning they do not present any noticeable signs or symptoms. For example, most individual infected with herpes simplex virus remain asymptomatic and are unaware that they have been infected.

Note:

- Explain the difference between signs and symptoms.

Classifications of Disease

The World Health Organization's (WHO) International Classification of Diseases (ICD) is used in clinical fields to classify diseases and monitor morbidity (the number of cases of a disease) and mortality (the number of deaths due to a disease). In this section, we will introduce terminology used by the ICD (and in health-care professions in general) to describe and categorize various types of disease.

An **infectious disease** is any disease caused by the direct effect of a pathogen. A pathogen may be cellular (bacteria, parasites, and fungi) or acellular (viruses, viroids, and prions). Some infectious diseases are also **communicable**, meaning they are capable of being spread from person to person through either direct or indirect mechanisms. Some infectious communicable diseases are also considered **contagious** diseases, meaning they are easily spread from person to person. Not all contagious diseases are equally so; the degree to which a disease is contagious usually depends on how the pathogen is transmitted. For example, measles is a highly contagious viral disease that can be transmitted when an infected person coughs or sneezes and an uninfected person breathes in droplets containing the virus. Gonorrhea is not as contagious as measles because transmission

of the pathogen (*Neisseria gonorrhoeae*) requires close intimate contact (usually sexual) between an infected person and an uninfected person.

Diseases that are contracted as the result of a medical procedure are known as **iatrogenic diseases**. Iatrogenic diseases can occur after procedures involving wound treatments, catheterization, or surgery if the wound or surgical site becomes contaminated. For example, an individual treated for a skin wound might acquire necrotizing fasciitis (an aggressive, “flesh-eating” disease) if bandages or other dressings became contaminated by *Clostridium perfringens* or one of several other bacteria that can cause this condition.

Diseases acquired in hospital settings are known as **nosocomial diseases**. Several factors contribute to the prevalence and severity of nosocomial diseases. First, sick patients bring numerous pathogens into hospitals, and some of these pathogens can be transmitted easily via improperly sterilized medical equipment, bed sheets, call buttons, door handles, or by clinicians, nurses, or therapists who do not wash their hands before touching a patient. Second, many hospital patients have weakened immune systems, making them more susceptible to infections. Compounding this, the prevalence of antibiotics in hospital settings can select for drug-resistant bacteria that can cause very serious infections that are difficult to treat.

Certain infectious diseases are not transmitted between humans directly but can be transmitted from animals to humans. Such a disease is called **zoonotic disease** (or **zoonosis**). According to WHO, a zoonosis is a disease that occurs when a pathogen is transferred from a vertebrate animal to a human; however, sometimes the term is defined more broadly to include diseases transmitted by all animals (including invertebrates). For example, rabies is a viral zoonotic disease spread from animals to humans through bites and contact with infected saliva. Many other zoonotic diseases rely on insects or other arthropods for transmission. Examples include yellow fever (transmitted through the bite of mosquitoes infected with yellow fever virus) and Rocky Mountain spotted fever (transmitted through the bite of ticks infected with *Rickettsia rickettsii*).

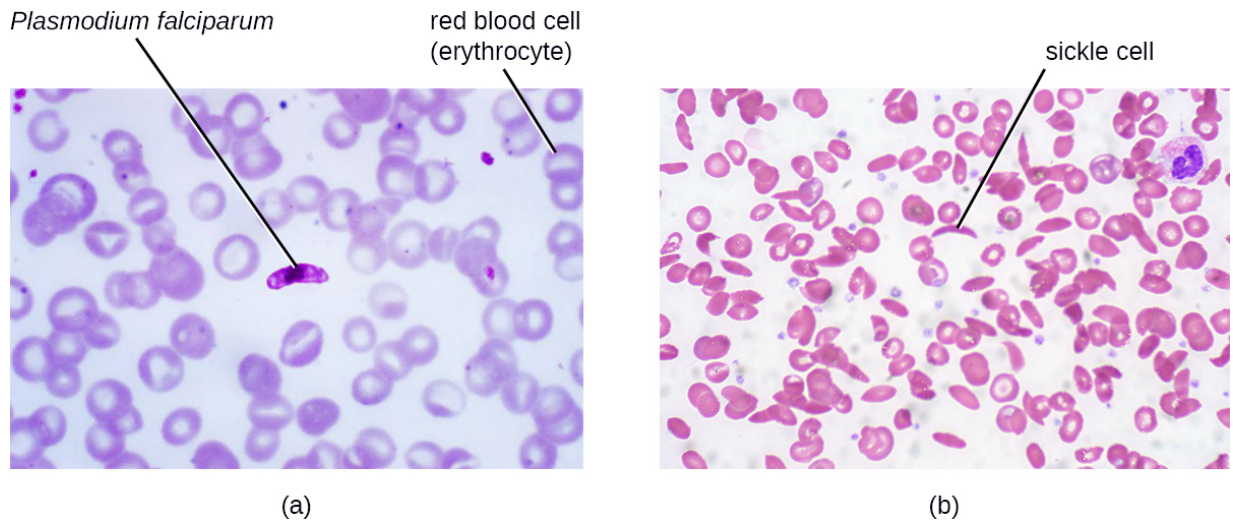
In contrast to communicable infectious diseases, a **noncommunicable** infectious disease is not spread from one person to another. One example is

tetanus, caused by *Clostridium tetani*, a bacterium that produces endospores that can survive in the soil for many years. This disease is typically only transmitted through contact with a skin wound; it cannot be passed from an infected person to another person. Similarly, Legionnaires disease is caused by *Legionella pneumophila*, a bacterium that lives within amoebae in moist locations like water-cooling towers. An individual may contract Legionnaires disease via contact with the contaminated water, but once infected, the individual cannot pass the pathogen to other individuals.

In addition to the wide variety of noncommunicable infectious diseases, **noninfectious diseases** (those not caused by pathogens) are an important cause of morbidity and mortality worldwide. Noninfectious diseases can be caused by a wide variety factors, including genetics, the environment, or immune system dysfunction, to name a few. For example, sickle cell anemia is an inherited disease caused by a genetic mutation that can be passed from parent to offspring ([\[link\]](#)). Other types of noninfectious diseases are listed in [\[link\]](#).

Types of Noninfectious Diseases		
Type	Definition	Example
Inherited	A genetic disease	Sickle cell anemia
Congenital	Disease that is present at or before birth	Down syndrome
Degenerative	Progressive, irreversible loss of function	Parkinson disease (affecting central nervous system)

Types of Noninfectious Diseases		
Type	Definition	Example
Nutritional deficiency	Impaired body function due to lack of nutrients	Scurvy (vitamin C deficiency)
Endocrine	Disease involving malfunction of glands that release hormones to regulate body functions	Hypothyroidism – thyroid does not produce enough thyroid hormone, which is important for metabolism
Neoplastic	Abnormal growth (benign or malignant)	Some forms of cancer
Idiopathic	Disease for which the cause is unknown	Idiopathic juxtafoveal retinal telangiectasia (dilated, twisted blood vessels in the retina of the eye)



Blood smears showing two diseases of the blood. (a) Malaria is an infectious, zoonotic disease caused by the protozoan pathogen *Plasmodium falciparum* (shown here) and several other species of the genus *Plasmodium*. It is transmitted by mosquitoes to humans. (b) Sickle cell disease is a noninfectious genetic disorder that results in abnormally shaped red blood cells, which can stick together and obstruct the flow of blood through the circulatory system. It is not caused by a pathogen, but rather a genetic mutation. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Ed Uthman)

Note:

Lists of common infectious diseases can be found at the following [Centers for Disease Control and Prevention](#) (CDC), [World Health Organization](#) (WHO), and [International Classification of Diseases](#) websites.

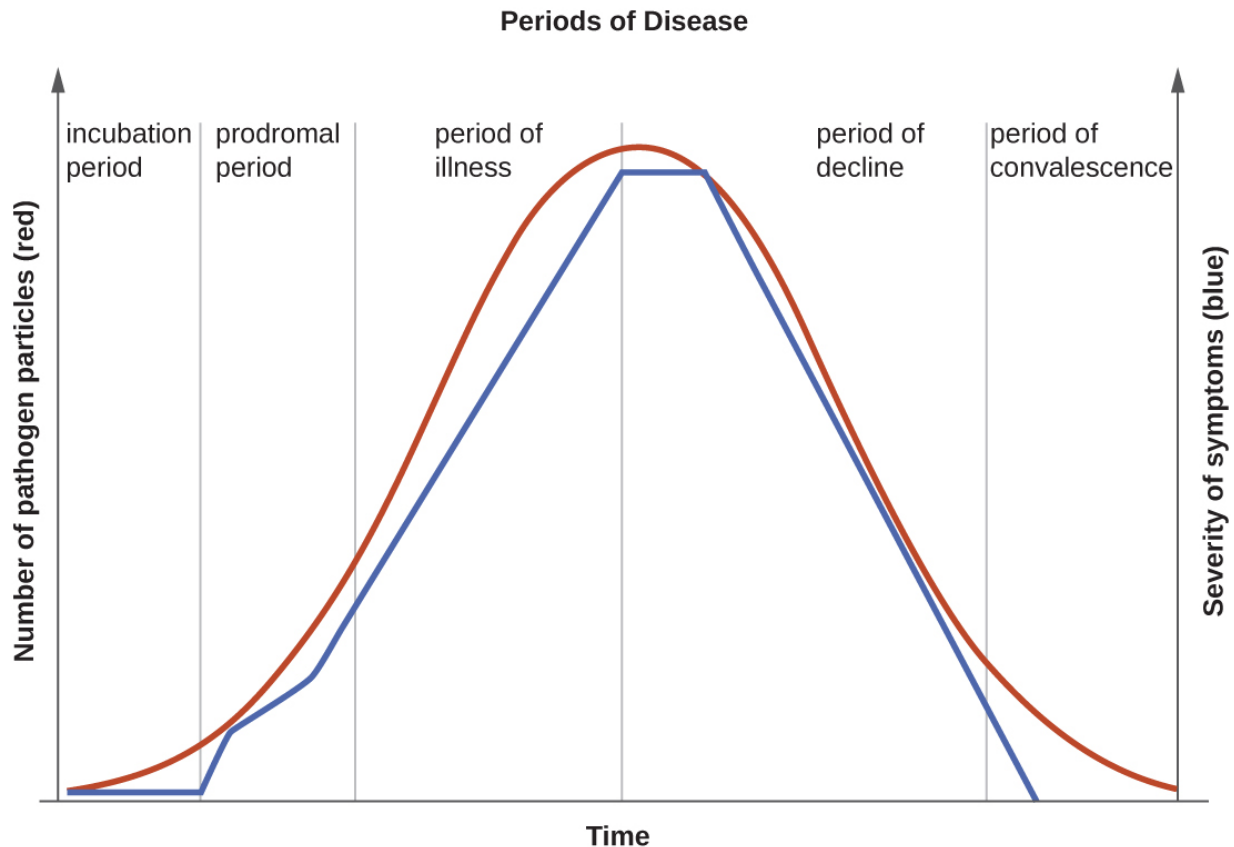
Note:

- Describe how a disease can be infectious but not contagious.

- Explain the difference between iatrogenic disease and nosocomial disease.

Periods of Disease

The five periods of disease (sometimes referred to as stages or phases) include the incubation, prodromal, illness, decline, and convalescence periods ([\[link\]](#)). The **incubation period** occurs in an acute disease after the initial entry of the pathogen into the host (patient). It is during this time the pathogen begins multiplying in the host. However, there are insufficient numbers of pathogen particles (cells or viruses) present to cause signs and symptoms of disease. Incubation periods can vary from a day or two in acute disease to months or years in chronic disease, depending upon the pathogen. Factors involved in determining the length of the incubation period are diverse, and can include strength of the pathogen, strength of the host immune defenses, site of infection, type of infection, and the size infectious dose received. During this incubation period, the patient is unaware that a disease is beginning to develop.



The progression of an infectious disease can be divided into five periods, which are related to the number of pathogen particles (red) and the severity of signs and symptoms (blue).

The **prodromal period** occurs after the incubation period. During this phase, the pathogen continues to multiply and the host begins to experience general signs and symptoms of illness, which typically result from activation of the immune system, such as fever, pain, soreness, swelling, or inflammation. Usually, such signs and symptoms are too general to indicate a particular disease. Following the prodromal period is the **period of illness**, during which the signs and symptoms of disease are most obvious and severe.

The period of illness is followed by the **period of decline**, during which the number of pathogen particles begins to decrease, and the signs and symptoms of illness begin to decline. However, during the decline period,

patients may become susceptible to developing secondary infections because their immune systems have been weakened by the primary infection. The final period is known as the **period of convalescence**. During this stage, the patient generally returns to normal functions, although some diseases may inflict permanent damage that the body cannot fully repair.

Infectious diseases can be contagious during all five of the periods of disease. Which periods of disease are more likely to be associated with transmissibility of an infection depends upon the disease, the pathogen, and the mechanisms by which the disease develops and progresses. For example, with meningitis (infection of the lining of brain), the periods of infectivity depend on the type of pathogen causing the infection. Patients with bacterial meningitis are contagious during the incubation period for up to a week before the onset of the prodromal period, whereas patients with viral meningitis become contagious when the first signs and symptoms of the prodromal period appear. With many viral diseases associated with rashes (e.g., chickenpox, measles, rubella, roseola), patients are contagious during the incubation period up to a week before the rash develops. In contrast, with many respiratory infections (e.g., colds, influenza, diphtheria, strep throat, and pertussis) the patient becomes contagious with the onset of the prodromal period. Depending upon the pathogen, the disease, and the individual infected, transmission can still occur during the periods of decline, convalescence, and even long after signs and symptoms of the disease disappear. For example, an individual recovering from a diarrheal disease may continue to carry and shed the pathogen in feces for some time, posing a risk of transmission to others through direct contact or indirect contact (e.g., through contaminated objects or food).

Note:

- Name some of the factors that can affect the length of the incubation period of a particular disease.

Acute and Chronic Diseases

The duration of the period of illness can vary greatly, depending on the pathogen, effectiveness of the immune response in the host, and any medical treatment received. For an **acute disease**, pathologic changes occur over a relatively short time (e.g., hours, days, or a few weeks) and involve a rapid onset of disease conditions. For example, influenza (caused by Influenzavirus) is considered an acute disease because the incubation period is approximately 1–2 days. Infected individuals can spread influenza to others for approximately 5 days after becoming ill. After approximately 1 week, individuals enter the period of decline.

For a **chronic disease**, pathologic changes can occur over longer time spans (e.g., months, years, or a lifetime). For example, chronic gastritis (inflammation of the lining of the stomach) is caused by the gram-negative bacterium *Helicobacter pylori*. *H. pylori* is able to colonize the stomach and persist in its highly acidic environment by producing the enzyme urease, which modifies the local acidity, allowing the bacteria to survive indefinitely.[\[footnote\]](#) Consequently, *H. pylori* infections can recur indefinitely unless the infection is cleared using antibiotics.[\[footnote\]](#) Hepatitis B virus can cause a chronic infection in some patients who do not eliminate the virus after the acute illness. A chronic infection with hepatitis B virus is characterized by the continued production of infectious virus for 6 months or longer after the acute infection, as measured by the presence of viral antigen in blood samples.

J.G. Kusters et al. Pathogenesis of *Helicobacter pylori* Infection. *Clinical Microbiology Reviews* 19 no. 3 (2006):449–490.

N.R. Salama et al. “Life in the Human Stomach: Persistence Strategies of the Bacterial Pathogen *Helicobacter pylori*.” *Nature Reviews Microbiology* 11 (2013):385–399.

In **latent diseases**, as opposed to chronic infections, the causal pathogen goes dormant for extended periods of time with no active replication. Examples of diseases that go into a latent state after the acute infection include herpes (herpes simplex viruses [HSV-1 and HSV-2]), chickenpox (varicella-zoster virus [VZV]), and mononucleosis (Epstein-Barr virus [EBV]). HSV-1, HSV-2, and VZV evade the host immune system by

residing in a latent form within cells of the nervous system for long periods of time, but they can reactivate to become active infections during times of stress and immunosuppression. For example, an initial infection by VZV may result in a case of childhood chickenpox, followed by a long period of latency. The virus may reactivate decades later, causing episodes of shingles in adulthood. EBV goes into latency in B cells of the immune system and possibly epithelial cells; it can reactivate years later to produce B-cell lymphoma.

Note:

- Explain the difference between latent disease and chronic disease.

Key Concepts and Summary

- In an **infection**, a microorganism enters a host and begins to multiply. Some infections cause **disease**, which is any deviation from the normal function or structure of the host.
- **Signs** of a disease are objective and are measured. **Symptoms** of a disease are subjective and are reported by the patient.
- Diseases can either be **noninfectious** (due to genetics and environment) or **infectious** (due to pathogens). Some infectious diseases are **communicable** (transmissible between individuals) or **contagious** (easily transmissible between individuals); others are **noncommunicable**, but may be contracted via contact with environmental reservoirs or animals (**zoonoses**)
- **Nosocomial diseases** are contracted in hospital settings, whereas **iatrogenic disease** are the direct result of a medical procedure
- An **acute disease** is short in duration, whereas a **chronic disease** lasts for months or years. **Latent diseases** last for years, but are distinguished from chronic diseases by the lack of active replication during extended dormant periods.

- The periods of disease include the **incubation period**, the **prodromal period**, the **period of illness**, the **period of decline**, and the **period of convalescence**. These periods are marked by changes in the number of infectious agents and the severity of signs and symptoms.

Multiple Choice

Exercise:

Problem: Which of the following would be a sign of an infection?

- A. muscle aches
- B. headache
- C. fever
- D. nausea

Solution:

C

Exercise:

Problem:

Which of the following is an example of a noncommunicable infectious disease?

- A. infection with a respiratory virus
- B. food poisoning due to a preformed bacterial toxin in food
- C. skin infection acquired from a dog bite
- D. infection acquired from the stick of a contaminated needle

Solution:

B

Exercise:

Problem:

During an oral surgery, the surgeon nicked the patient's gum with a sharp instrument. This allowed *Streptococcus*, a bacterium normally present in the mouth, to gain access to the blood. As a result, the patient developed bacterial endocarditis (an infection of the heart). Which type of disease is this?

- A. iatrogenic
- B. nosocomial
- C. vectors
- D. zoonotic

Solution:

A

Exercise:**Problem:**

Which period is the stage of disease during which the patient begins to present general signs and symptoms?

- A. convalescence
- B. incubation
- C. illness
- D. prodromal

Solution:

D

Exercise:

Problem:

A communicable disease that can be easily transmitted from person to person is which type of disease?

- A. contagious
- B. iatrogenic
- C. acute
- D. nosocomial

Solution:

A

Fill in the Blank**Exercise:****Problem:**

A difference between an acute disease and chronic disease is that chronic diseases have an extended period of _____.

Solution:

illness

Exercise:**Problem:**

A person steps on a rusty nail and develops tetanus. In this case, the person has acquired a(n) _____ disease.

Solution:

noncommunicable

Short Answer

Exercise:

Problem:

Brian goes to the hospital after not feeling well for a week. He has a fever of 38 °C (100.4 °F) and complains of nausea and a constant migraine. Distinguish between the signs and symptoms of disease in Brian's case.

Critical Thinking

Exercise:

Problem:

Two periods of acute disease are the periods of illness and period of decline. (a) In what way are both of these periods similar? (b) In terms of quantity of pathogen, in what way are these periods different? (c) What initiates the period of decline?

Exercise:

Problem:

In July 2015, a report (C. Owens. "*P. aeruginosa* survives in sinks 10 years after hospital outbreak." 2015. <http://www.healio.com/infectious-disease/nosocomial-infections/news/online/%7B5afba909-56d9-48cc-a9b0-ffe4568161e8%7D/p-aeruginosa-survives-in-sinks-10-years-after-hospital-outbreak>) was released indicating the gram-negative bacterium *Pseudomonas aeruginosa* was found on hospital sinks 10 years after the initial outbreak in a neonatal intensive care unit. *P. aeruginosa* usually causes localized ear and eye infections but can cause pneumonia or septicemia in vulnerable individuals like newborn babies. Explain how the current discovery of the presence of this reported *P. aeruginosa* could lead to a recurrence of nosocomial disease.

How Pathogens Cause Disease

LEARNING OBJECTIVES

- Summarize Koch's postulates and molecular Koch's postulates, respectively, and explain their significance and limitations
- Explain the concept of pathogenicity (virulence) in terms of infectious and lethal dose
- Distinguish between primary and opportunistic pathogens and identify specific examples of each
- Summarize the stages of pathogenesis
- Explain the roles of portals of entry and exit in the transmission of disease and identify specific examples of these portals

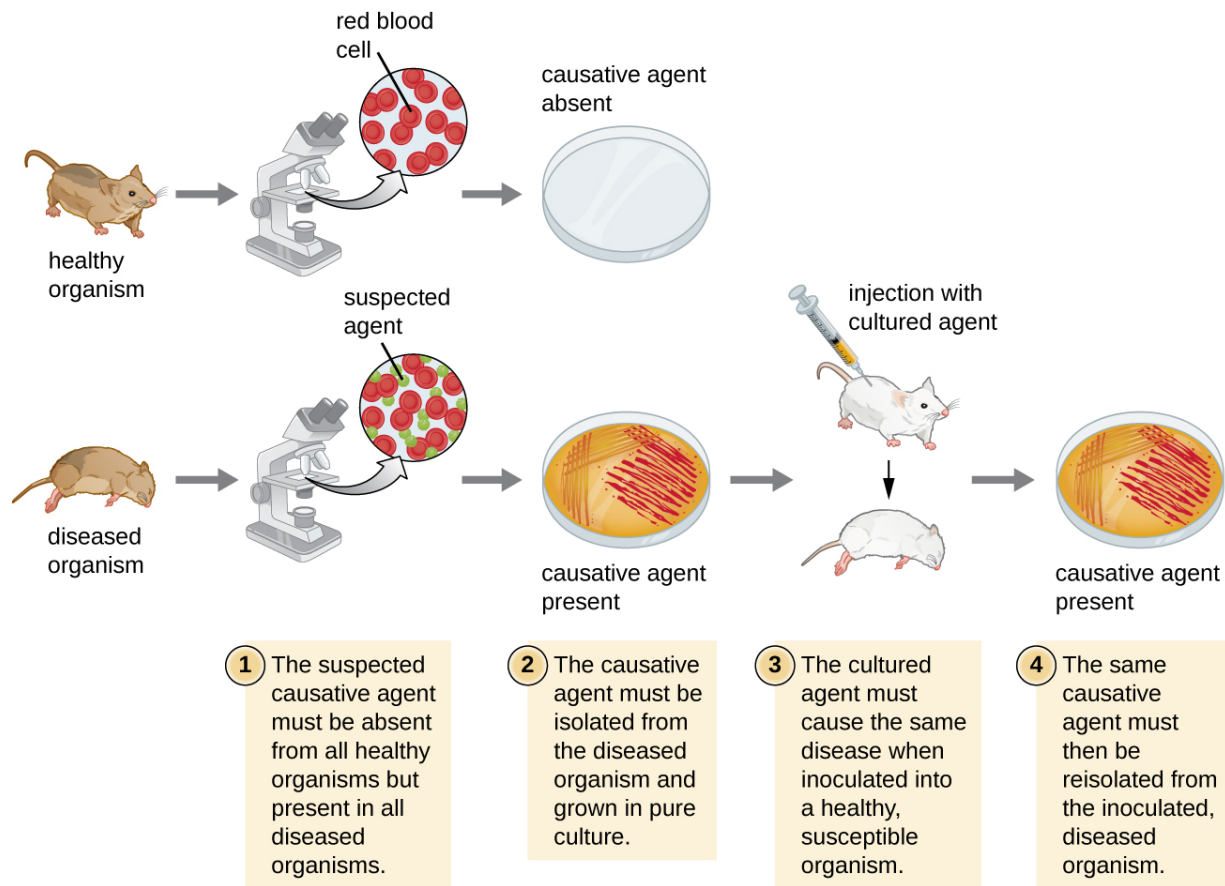
For most infectious diseases, the ability to accurately identify the causative pathogen is a critical step in finding or prescribing effective treatments. Today's physicians, patients, and researchers owe a sizable debt to the physician Robert Koch (1843–1910), who devised a systematic approach for confirming causative relationships between diseases and specific pathogens.

Koch's Postulates

In 1884, Koch published four postulates ([\[link\]](#)) that summarized his method for determining whether a particular microorganism was the cause of a particular disease. Each of Koch's postulates represents a criterion that must be met before a disease can be positively linked with a pathogen. In

order to determine whether the criteria are met, tests are performed on laboratory animals and cultures from healthy and diseased animals are compared ([link](#)).

Koch's Postulates
(1) The suspected pathogen must be found in every case of disease and not be found in healthy individuals.
(2) The suspected pathogen can be isolated and grown in pure culture.
(3) A healthy test subject infected with the suspected pathogen must develop the same signs and symptoms of disease as seen in postulate 1.
(4) The pathogen must be re-isolated from the new host and must be identical to the pathogen from postulate 2.



The steps for confirming that a pathogen is the cause of a particular disease using Koch's postulates.

In many ways, Koch's postulates are still central to our current understanding of the causes of disease. However, advances in microbiology have revealed some important limitations in Koch's criteria. Koch made several assumptions that we now know are untrue in many cases. The first relates to postulate 1, which assumes that pathogens are only found in diseased, not healthy, individuals. This is not true for many pathogens. For example, *H. pylori*, described earlier in this chapter as a pathogen causing chronic gastritis, is also part of the normal microbiota of the stomach in many healthy humans who never develop gastritis. It is estimated that upwards of 50% of the human population acquires *H. pylori* early in life, with most maintaining it as part of the normal microbiota for the rest of their life without ever developing disease.

Koch's second faulty assumption was that all healthy test subjects are equally susceptible to disease. We now know that individuals are not equally susceptible to disease. Individuals are unique in terms of their microbiota and the state of their immune system at any given time. The makeup of the resident microbiota can influence an individual's susceptibility to an infection. Members of the normal microbiota play an important role in immunity by inhibiting the growth of transient pathogens. In some cases, the microbiota may prevent a pathogen from establishing an infection; in others, it may not prevent an infection altogether but may influence the severity or type of signs and symptoms. As a result, two individuals with the same disease may not always present with the same signs and symptoms. In addition, some individuals have stronger immune systems than others. Individuals with immune systems weakened by age or an unrelated illness are much more susceptible to certain infections than individuals with strong immune systems.

Koch also assumed that all pathogens are microorganisms that can be grown in pure culture (postulate 2) and that animals could serve as reliable models for human disease. However, we now know that not all pathogens can be grown in pure culture, and many human diseases cannot be reliably replicated in animal hosts. Viruses and certain bacteria, including *Rickettsia* and *Chlamydia*, are obligate intracellular pathogens that can grow only when inside a host cell. If a microbe cannot be cultured, a researcher cannot move past postulate 2. Likewise, without a suitable nonhuman host, a researcher cannot evaluate postulate 2 without deliberately infecting humans, which presents obvious ethical concerns. AIDS is an example of such a disease because the human immunodeficiency virus (HIV) only causes disease in humans.

Note:

- Briefly summarize the limitations of Koch's postulates.

Molecular Koch’s Postulates

In 1988, Stanley Falkow (1934–) proposed a revised form of Koch’s postulates known as molecular Koch’s postulates. These are listed in the left column of [\[link\]](#). The premise for molecular Koch’s postulates is not in the ability to isolate a particular pathogen but rather to identify a gene that may cause the organism to be pathogenic.

Falkow’s modifications to Koch’s original postulates explain not only infections caused by intracellular pathogens but also the existence of pathogenic strains of organisms that are usually nonpathogenic. For example, the predominant form of the bacterium *Escherichia coli* is a member of the normal microbiota of the human intestine and is generally considered harmless. However, there are pathogenic strains of *E. coli* such as enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (O157:H7) (EHEC). We now know ETEC and EHEC exist because of the acquisition of new genes by the once-harmless *E. coli*, which, in the form of these pathogenic strains, is now capable of producing toxins and causing illness. The pathogenic forms resulted from minor genetic changes. The right-side column of [\[link\]](#) illustrates how molecular Koch’s postulates can be applied to identify EHEC as a pathogenic bacterium.

Molecular Koch’s Postulates Applied to EHEC	
Molecular Koch’s Postulates	Application to EHEC
(1) The phenotype (sign or symptom of disease) should be associated only with pathogenic strains of a species.	EHEC causes intestinal inflammation and diarrhea, whereas nonpathogenic strains of <i>E. coli</i> do not.

Molecular Koch's Postulates Applied to EHEC	
Molecular Koch's Postulates	Application to EHEC
(2) Inactivation of the suspected gene(s) associated with pathogenicity should result in a measurable loss of pathogenicity.	One of the genes in EHEC encodes for Shiga toxin, a bacterial toxin (poison) that inhibits protein synthesis. Inactivating this gene reduces the bacteria's ability to cause disease.
(3) Reversion of the inactive gene should restore the disease phenotype.	By adding the gene that encodes the toxin back into the genome (e.g., with a phage or plasmid), EHEC's ability to cause disease is restored.

As with Koch's original postulates, the molecular Koch's postulates have limitations. For example, genetic manipulation of some pathogens is not possible using current methods of molecular genetics. In a similar vein, some diseases do not have suitable animal models, which limits the utility of both the original and molecular postulates.

Note:

- Explain the differences between Koch's original postulates and the molecular Koch's postulates.

Pathogenicity and Virulence

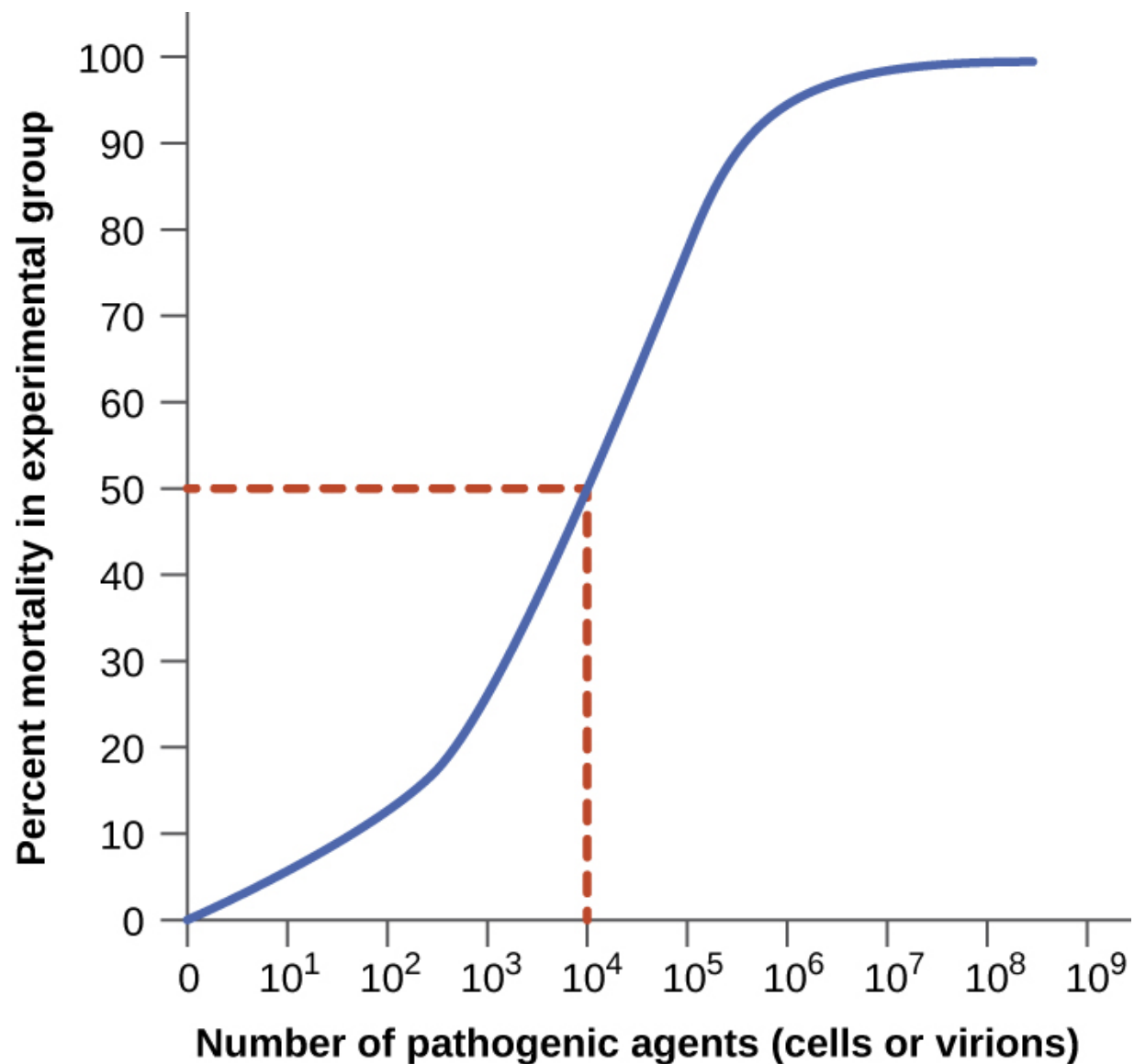
The ability of a microbial agent to cause disease is called **pathogenicity**, and the degree to which an organism is pathogenic is called **virulence**.

Virulence is a continuum. On one end of the spectrum are organisms that are avirulent (not harmful) and on the other are organisms that are highly virulent. Highly virulent pathogens will almost always lead to a disease state when introduced to the body, and some may even cause multi-organ and body system failure in healthy individuals. Less virulent pathogens may cause an initial infection, but may not always cause severe illness. Pathogens with low virulence would more likely result in mild signs and symptoms of disease, such as low-grade fever, headache, or muscle aches. Some individuals might even be asymptomatic.

An example of a highly virulent microorganism is *Bacillus anthracis*, the pathogen responsible for anthrax. *B. anthracis* can produce different forms of disease, depending on the route of transmission (e.g., cutaneous injection, inhalation, ingestion). The most serious form of anthrax is inhalation anthrax. After *B. anthracis* spores are inhaled, they germinate. An active infection develops and the bacteria release potent toxins that cause edema (fluid buildup in tissues), hypoxia (a condition preventing oxygen from reaching tissues), and necrosis (cell death and inflammation). Signs and symptoms of inhalation anthrax include high fever, difficulty breathing, vomiting and coughing up blood, and severe chest pains suggestive of a heart attack. With inhalation anthrax, the toxins and bacteria enter the bloodstream, which can lead to multi-organ failure and death of the patient. If a gene (or genes) involved in pathogenesis is inactivated, the bacteria become less virulent or nonpathogenic.

Virulence of a pathogen can be quantified using controlled experiments with laboratory animals. Two important indicators of virulence are the **median infectious dose (ID₅₀)** and the **median lethal dose (LD₅₀)**, both of which are typically determined experimentally using animal models. The ID₅₀ is the number of pathogen cells or virions required to cause active infection in 50% of inoculated animals. The LD₅₀ is the number of pathogenic cells, virions, or amount of toxin required to kill 50% of infected animals. To calculate these values, each group of animals is inoculated with one of a range of known numbers of pathogen cells or virions. In graphs like the one shown in [\[link\]](#), the percentage of animals that have been infected (for ID₅₀) or killed (for LD₅₀) is plotted against the concentration of pathogen inoculated. [\[link\]](#) represents data graphed from a

hypothetical experiment measuring the LD₅₀ of a pathogen. Interpretation of the data from this graph indicates that the LD₅₀ of the pathogen for the test animals is 10⁴ pathogen cells or virions (depending upon the pathogen studied).



A graph like this is used to determine LD₅₀ by plotting pathogen concentration against the percent of infected test animals that have died. In this example, the LD₅₀ = 10⁴ pathogenic particles.

[\[link\]](#) lists selected foodborne pathogens and their ID₅₀ values in humans (as determined from epidemiologic data and studies on human volunteers). Keep in mind that these are *median* values. The actual infective dose for an individual can vary widely, depending on factors such as route of entry; the age, health, and immune status of the host; and environmental and pathogen-specific factors such as susceptibility to the acidic pH of the stomach. It is also important to note that a pathogen's infective dose does not necessarily correlate with disease severity. For example, just a single cell of *Salmonella enterica* serotype Typhimurium can result in an active infection. The resultant disease, *Salmonella* gastroenteritis or salmonellosis, can cause nausea, vomiting, and diarrhea, but has a mortality rate of less than 1% in healthy adults. In contrast, *S. enterica* serotype Typhi has a much higher ID₅₀, typically requiring as many as 1,000 cells to produce infection. However, this serotype causes typhoid fever, a much more systemic and severe disease that has a mortality rate as high as 10% in untreated individuals.

ID₅₀ for Selected Foodborne Diseases [footnote] Food and Drug Administration. “Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins.” 2nd ed. Silver Spring, MD: US Food and Drug Administration; 2012.	
Pathogen	ID₅₀
Viruses	
Hepatitis A virus	10–100
Norovirus	1–10
Rotavirus	10–100

ID₅₀ for Selected Foodborne Diseases[\[footnote\]](#)

Food and Drug Administration. “Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins.” 2nd ed. Silver Spring, MD: US Food and Drug Administration; 2012.

Pathogen	ID ₅₀
Bacteria	
<i>Escherichia coli</i> , enterohemorrhagic (EHEC, serotype O157)	10–100
<i>E. coli</i> , enteroinvasive (EIEC)	200–5,000
<i>E. coli</i> , enteropathogenic (EPEC)	10,000,000– 10,000,000,000
<i>E. coli</i> , enterotoxigenic (ETEC)	10,000,000– 10,000,000,000
<i>Salmonella enterica</i> serovar Typhi	<1,000
<i>S. enterica</i> serovar Typhimurium	≥1
<i>Shigella dysenteriae</i>	10–200
<i>Vibrio cholerae</i> (serotypes O139, O1)	1,000,000
<i>V. parahemolyticus</i>	100,000,000
Protozoa	
<i>Giardia lamblia</i>	1
<i>Cryptosporidium parvum</i>	10–100

Note:

- What is the difference between a pathogen's infective dose and lethal dose?
- Which is more closely related to the severity of a disease?

Primary Pathogens versus Opportunistic Pathogens

Pathogens can be classified as either primary pathogens or opportunistic pathogens. A **primary pathogen** can cause disease in a host regardless of the host's resident microbiota or immune system. An **opportunistic pathogen**, by contrast, can only cause disease in situations that compromise the host's defenses, such as the body's protective barriers, immune system, or normal microbiota. Individuals susceptible to opportunistic infections include the very young, the elderly, women who are pregnant, patients undergoing chemotherapy, people with immunodeficiencies (such as acquired immunodeficiency syndrome [AIDS]), patients who are recovering from surgery, and those who have had a breach of protective barriers (such as a severe wound or burn).

An example of a primary pathogen is enterohemorrhagic *E. coli* (EHEC), which produces a virulence factor known as Shiga toxin. This toxin inhibits protein synthesis, leading to severe and bloody diarrhea, inflammation, and renal failure, even in patients with healthy immune systems. *Staphylococcus epidermidis*, on the other hand, is an opportunistic pathogen that is among the most frequent causes of nosocomial disease.[\[footnote\]](#) *S. epidermidis* is a member of the normal microbiota of the skin, where it is generally avirulent. However, in hospitals, it can also grow in biofilms that form on catheters, implants, or other devices that are inserted into the body during surgical procedures. Once inside the body, *S. epidermidis* can cause serious infections such as endocarditis, and it produces virulence factors that promote the persistence of such infections.

M. Otto. "Staphylococcus epidermidis--The 'Accidental' Pathogen." *Nature Reviews Microbiology* 7 no. 8 (2009):555–567.

Other members of the normal microbiota can also cause opportunistic infections under certain conditions. This often occurs when microbes that reside harmlessly in one body location end up in a different body system, where they cause disease. For example, *E. coli* normally found in the large intestine can cause a urinary tract infection if it enters the bladder. This is the leading cause of urinary tract infections among women.

Members of the normal microbiota may also cause disease when a shift in the environment of the body leads to overgrowth of a particular microorganism. For example, the yeast *Candida* is part of the normal microbiota of the skin, mouth, intestine, and vagina, but its population is kept in check by other organisms of the microbiota. If an individual is taking antibacterial medications, however, bacteria that would normally inhibit the growth of *Candida* can be killed off, leading to a sudden growth in the population of *Candida*, which is not affected by antibacterial medications because it is a fungus. An overgrowth of *Candida* can manifest as oral thrush (growth on mouth, throat, and tongue), a vaginal yeast infection, or cutaneous candidiasis. Other scenarios can also provide opportunities for *Candida* infections. Untreated diabetes can result in a high concentration of glucose in the saliva, which provides an optimal environment for the growth of *Candida*, resulting in thrush. Immunodeficiencies such as those seen in patients with HIV, AIDS, and cancer also lead to higher incidence of thrush. Vaginal yeast infections can result from decreases in estrogen levels during the menstruation or menopause. The amount of glycogen available to lactobacilli in the vagina is controlled by levels of estrogen; when estrogen levels are low, lactobacilli produce less lactic acid. The resultant increase in vaginal pH allows overgrowth of *Candida* in the vagina.

Note:

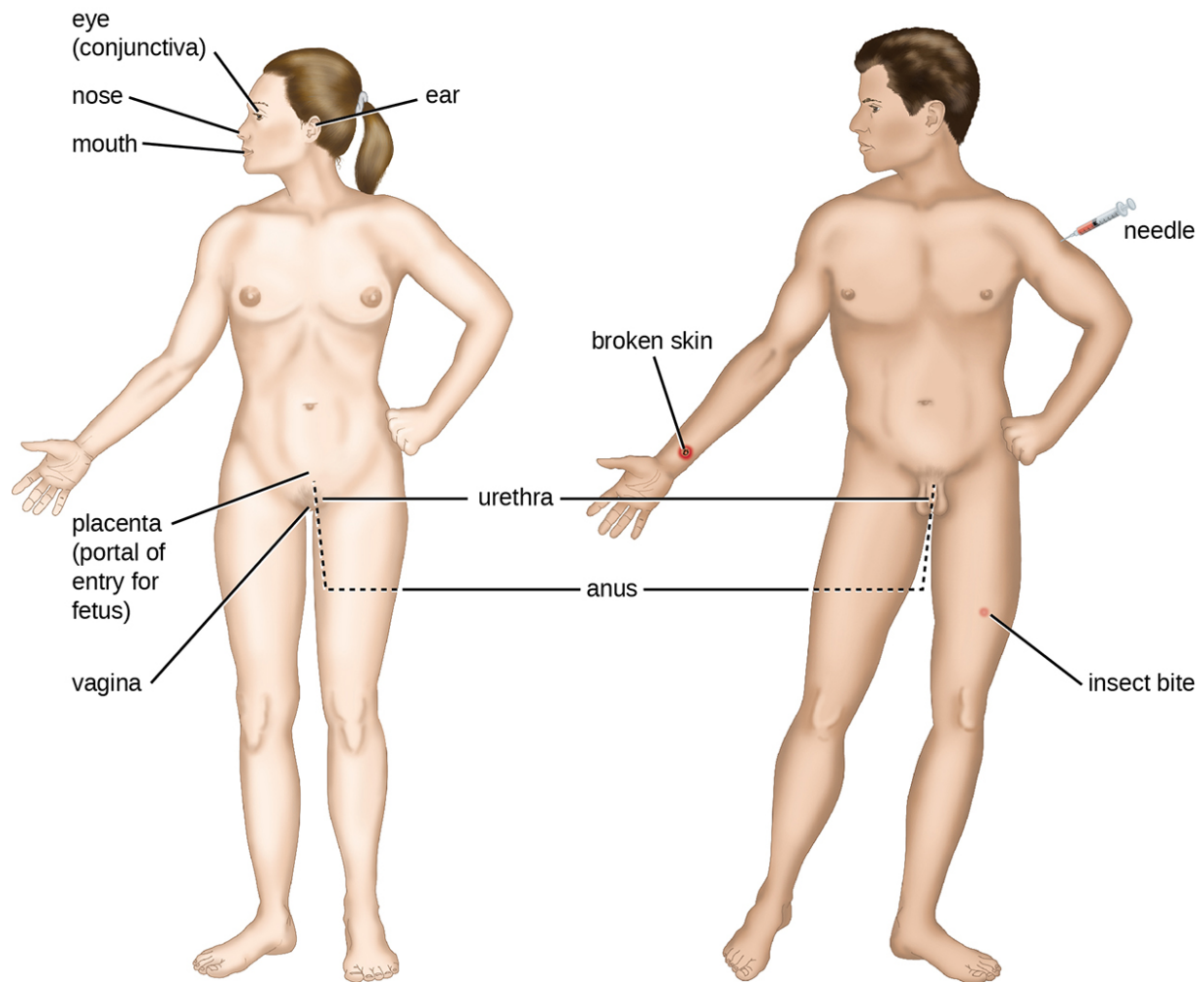
- Explain the difference between a primary pathogen and an opportunistic pathogen.
- Describe some conditions under which an opportunistic infection can occur.

Stages of Pathogenesis

To cause disease, a pathogen must successfully achieve four steps or stages of pathogenesis: exposure (contact), adhesion (colonization), invasion, and infection. The pathogen must be able to gain entry to the host, travel to the location where it can establish an infection, evade or overcome the host's immune response, and cause damage (i.e., disease) to the host. In many cases, the cycle is completed when the pathogen exits the host and is transmitted to a new host.

Exposure

An encounter with a potential pathogen is known as **exposure** or **contact**. The food we eat and the objects we handle are all ways that we can come into contact with potential pathogens. Yet, not all contacts result in infection and disease. For a pathogen to cause disease, it needs to be able to gain access into host tissue. An anatomic site through which pathogens can pass into host tissue is called a **portal of entry**. These are locations where the host cells are in direct contact with the external environment. Major portals of entry are identified in [\[link\]](#) and include the skin, mucous membranes, and parenteral routes.



Shown are different portals of entry where pathogens can gain access into the body. With the exception of the placenta, many of these locations are directly exposed to the external environment.

Mucosal surfaces are the most important portals of entry for microbes; these include the mucous membranes of the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Although most mucosal surfaces are in the interior of the body, some are contiguous with the external skin at various body openings, including the eyes, nose, mouth, urethra, and anus.

Most pathogens are suited to a particular portal of entry. A pathogen's portal specificity is determined by the organism's environmental adaptations

and by the enzymes and toxins they secrete. The respiratory and gastrointestinal tracts are particularly vulnerable portals of entry because particles that include microorganisms are constantly inhaled or ingested, respectively.

Pathogens can also enter through a breach in the protective barriers of the skin and mucous membranes. Pathogens that enter the body in this way are said to enter by the **parenteral route**. For example, the skin is a good natural barrier to pathogens, but breaks in the skin (e.g., wounds, insect bites, animal bites, needle pricks) can provide a parenteral portal of entry for microorganisms.

In pregnant women, the placenta normally prevents microorganisms from passing from the mother to the fetus. However, a few pathogens are capable of crossing the blood-placental barrier. The gram-positive bacterium *Listeria monocytogenes*, which causes the foodborne disease listeriosis, is one example that poses a serious risk to the fetus and can sometimes lead to spontaneous abortion. Other pathogens that can pass the placental barrier to infect the fetus are known collectively by the acronym TORCH ([link](#)).

Transmission of infectious diseases from mother to baby is also a concern at the time of birth when the baby passes through the birth canal. Babies whose mothers have active chlamydia or gonorrhea infections may be exposed to the causative pathogens in the vagina, which can result in eye infections that lead to blindness. To prevent this, it is standard practice to administer antibiotic drops to infants’ eyes shortly after birth.

Pathogens Capable of Crossing the Placental Barrier (TORCH Infections)		
	Disease	Pathogen

Pathogens Capable of Crossing the Placental Barrier (TORCH Infections)		
	Disease	Pathogen
T	Toxoplasmosis	<i>Toxoplasma gondii</i> (protozoan)
O [footnote] The O in TORCH stands for “other.”	Syphilis Chickenpox Hepatitis B HIV Fifth disease (erythema infectiosum)	<i>Treponema pallidum</i> (bacterium) Varicella-zoster virus (human herpesvirus 3) Hepatitis B virus (hepadnavirus) Retrovirus Parvovirus B19
R	Rubella (German measles)	Togavirus
C	Cytomegalovirus	Human herpesvirus 5
H	Herpes	Herpes simplex viruses (HSV) 1 and 2

Note:

Part 2

At the clinic, a physician takes down Michael’s medical history and asks about his activities and diet over the past week. Upon learning that Michael became sick the day after the party, the physician orders a blood test to check for pathogens associated with foodborne diseases. After tests confirm that presence of a gram-positive rod in Michael’s blood, he is given an injection of a broad-spectrum antibiotic and sent to a nearby

hospital, where he is admitted as a patient. There he is to receive additional intravenous antibiotic therapy and fluids.

- Is this bacterium in Michael's blood part of normal microbiota?
- What portal of entry did the bacteria use to cause this infection?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

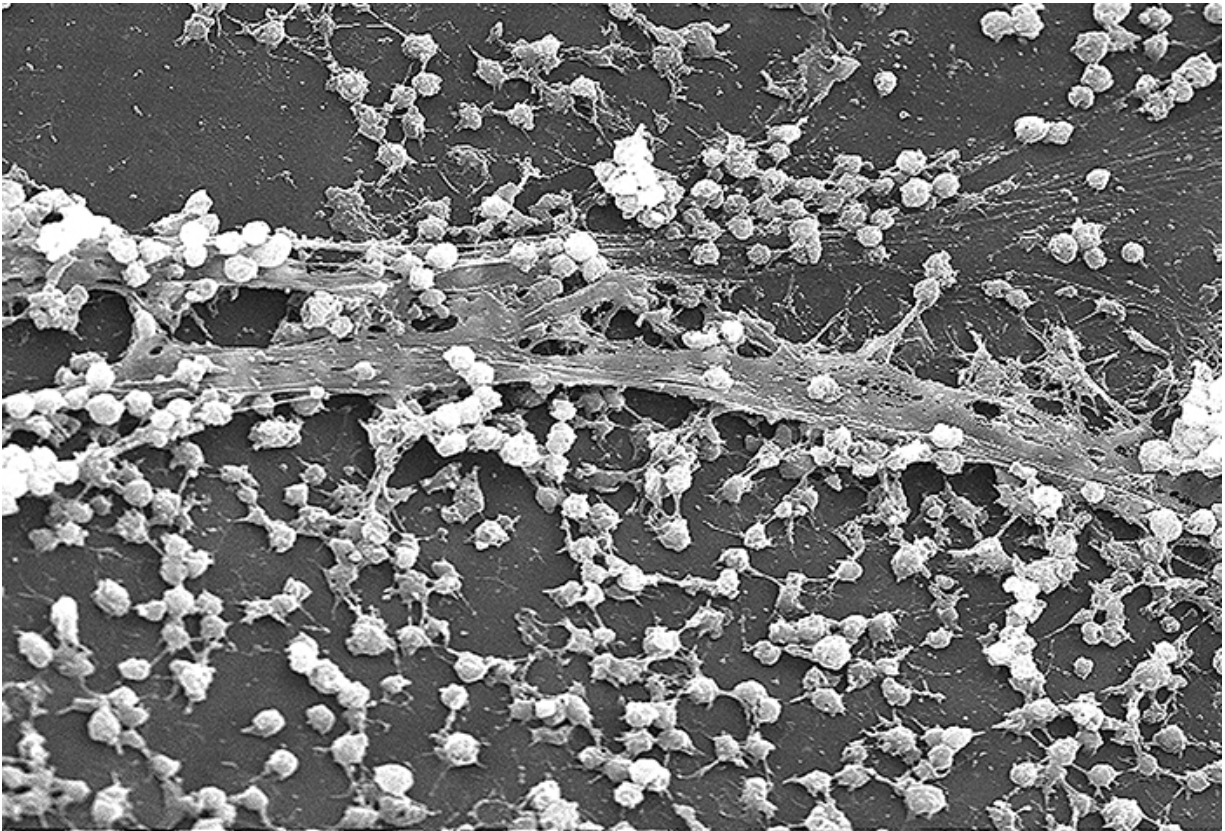
Adhesion

Following the initial exposure, the pathogen adheres at the portal of entry. The term **adhesion** refers to the capability of pathogenic microbes to attach to the cells of the body using adhesion factors, and different pathogens use various mechanisms to adhere to the cells of host tissues.

Molecules (either proteins or carbohydrates) called **adhesins** are found on the surface of certain pathogens and bind to specific receptors (glycoproteins) on host cells. Adhesins are present on the fimbriae and flagella of bacteria, the cilia of protozoa, and the capsids or membranes of viruses. Protozoans can also use hooks and barbs for adhesion; spike proteins on viruses also enhance viral adhesion. The production of glycocalyxes (slime layers and capsules) ([link](#)), with their high sugar and protein content, can also allow certain bacterial pathogens to attach to cells.

Biofilm growth can also act as an adhesion factor. A biofilm is a community of bacteria that produce a glycocalyx, known as extrapolymeric substance (EPS), that allows the biofilm to attach to a surface. Persistent *Pseudomonas aeruginosa* infections are common in patients suffering from cystic fibrosis, burn wounds, and middle-ear infections (otitis media) because *P. aeruginosa* produces a biofilm. The EPS allows the bacteria to adhere to the host cells and makes it harder for the host to physically remove the pathogen. The EPS not only allows for attachment but provides protection against the immune system and antibiotic treatments, preventing antibiotics from reaching the bacterial cells within the biofilm. In addition, not all bacteria in a biofilm are rapidly growing; some are in stationary

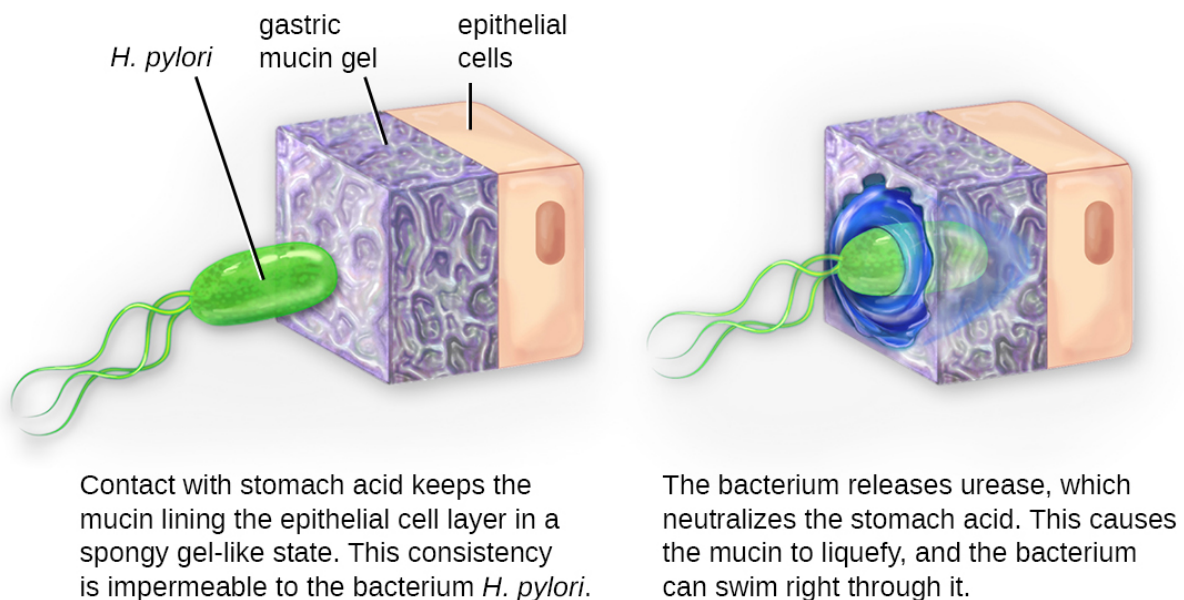
phase. Since antibiotics are most effective against rapidly growing bacteria, portions of bacteria in a biofilm are protected against antibiotics. [\[footnote\]](#)
D. Davies. “Understanding Biofilm Resistance to Antibacterial Agents.”
Nature Reviews Drug Discovery 2 (2003):114–122.



Glycocalyx produced by bacteria in a biofilm allows the cells to adhere to host tissues and to medical devices such as the catheter surface shown here. (credit: modification of work by Centers for Disease Control and Prevention)

Invasion

Once adhesion is successful, **invasion** can proceed. Invasion involves the dissemination of a pathogen throughout local tissues or the body. Pathogens may produce exoenzymes or toxins, which serve as virulence factors that allow them to colonize and damage host tissues as they spread deeper into the body. Pathogens may also produce virulence factors that protect them against immune system defenses. A pathogen's specific virulence factors determine the degree of tissue damage that occurs. [\[link\]](#) shows the invasion of *H. pylori* into the tissues of the stomach, causing damage as it progresses.



H. pylori is able to invade the lining of the stomach by producing virulence factors that enable it pass through the mucin layer covering epithelial cells. (credit: modification of work by Zina Deretsky, National Science Foundation)

Intracellular pathogens achieve invasion by entering the host's cells and reproducing. Some are obligate intracellular pathogens (meaning they can only reproduce inside of host cells) and others are facultative intracellular pathogens (meaning they can reproduce either inside or outside of host

cells). By entering the host cells, intracellular pathogens are able to evade some mechanisms of the immune system while also exploiting the nutrients in the host cell.

Entry to a cell can occur by endocytosis. For most kinds of host cells, pathogens use one of two different mechanisms for endocytosis and entry. One mechanism relies on effector proteins secreted by the pathogen; these effector proteins trigger entry into the host cell. This is the method that *Salmonella* and *Shigella* use when invading intestinal epithelial cells. When these pathogens come in contact with epithelial cells in the intestine, they secrete effector molecules that cause protrusions of membrane ruffles that bring the bacterial cell in. This process is called membrane ruffling. The second mechanism relies on surface proteins expressed on the pathogen that bind to receptors on the host cell, resulting in entry. For example, *Yersinia pseudotuberculosis* produces a surface protein known as invasin that binds to beta-1 integrins expressed on the surface of host cells.

Some host cells, such as white blood cells and other phagocytes of the immune system, actively endocytose pathogens in a process called phagocytosis. Although phagocytosis allows the pathogen to gain entry to the host cell, in most cases, the host cell kills and degrades the pathogen by using digestive enzymes. Normally, when a pathogen is ingested by a phagocyte, it is enclosed within a phagosome in the cytoplasm; the phagosome fuses with a lysosome to form a phagolysosome, where digestive enzymes kill the pathogen (see [Pathogen Recognition and Phagocytosis](#)). However, some intracellular pathogens have the ability to survive and multiply within phagocytes. Examples include *Listeria monocytogenes* and *Shigella*; these bacteria produce proteins that lyse the phagosome before it fuses with the lysosome, allowing the bacteria to escape into the phagocyte's cytoplasm where they can multiply. Bacteria such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Salmonella* species use a slightly different mechanism to evade being digested by the phagocyte. These bacteria prevent the fusion of the phagosome with the lysosome, thus remaining alive and dividing within the phagosome.

Infection

Following invasion, successful multiplication of the pathogen leads to infection. Infections can be described as local, focal, or systemic, depending on the extent of the infection. A **local infection** is confined to a small area of the body, typically near the portal of entry. For example, a hair follicle infected by *Staphylococcus aureus* infection may result in a boil around the site of infection, but the bacterium is largely contained to this small location. Other examples of local infections that involve more extensive tissue involvement include urinary tract infections confined to the bladder or pneumonia confined to the lungs.

In a **focal infection**, a localized pathogen, or the toxins it produces, can spread to a secondary location. For example, a dental hygienist nicking the gum with a sharp tool can lead to a local infection in the gum by *Streptococcus* bacteria of the normal oral microbiota. These *Streptococcus* spp. may then gain access to the bloodstream and make their way to other locations in the body, resulting in a secondary infection.

When an infection becomes disseminated throughout the body, we call it a **systemic infection**. For example, infection by the varicella-zoster virus typically gains entry through a mucous membrane of the upper respiratory system. It then spreads throughout the body, resulting in the classic red skin lesions associated with chickenpox. Since these lesions are not sites of initial infection, they are signs of a systemic infection.

Sometimes a **primary infection**, the initial infection caused by one pathogen, can lead to a **secondary infection** by another pathogen. For example, the immune system of a patient with a primary infection by HIV becomes compromised, making the patient more susceptible to secondary diseases like oral thrush and others caused by opportunistic pathogens. Similarly, a primary infection by Influenzavirus damages and decreases the defense mechanisms of the lungs, making patients more susceptible to a secondary pneumonia by a bacterial pathogen like *Haemophilus influenzae* or *Streptococcus pneumoniae*. Some secondary infections can even develop as a result of treatment for a primary infection. Antibiotic therapy targeting the primary pathogen can cause collateral damage to the normal microbiota,

creating an opening for opportunistic pathogens (see [Case in Point: A Secondary Yeast Infection](#)).

Note:

A Secondary Yeast Infection

Anita, a 36-year-old mother of three, goes to an urgent care center complaining of pelvic pressure, frequent and painful urination, abdominal cramps, and occasional blood-tinged urine. Suspecting a urinary tract infection (UTI), the physician requests a urine sample and sends it to the lab for a urinalysis. Since it will take approximately 24 hours to get the results of the culturing, the physician immediately starts Anita on the antibiotic ciprofloxacin. The next day, the microbiology lab confirms the presence of *E. coli* in Anita's urine, which is consistent with the presumptive diagnosis. However, the antimicrobial susceptibility test indicates that ciprofloxacin would not effectively treat Anita's UTI, so the physician prescribes a different antibiotic.

After taking her antibiotics for 1 week, Anita returns to the clinic complaining that the prescription is not working. Although the painful urination has subsided, she is now experiencing vaginal itching, burning, and discharge. After a brief examination, the physician explains to Anita that the antibiotics were likely successful in killing the *E. coli* responsible for her UTI; however, in the process, they also wiped out many of the "good" bacteria in Anita's normal microbiota. The new symptoms that Anita has reported are consistent with a secondary yeast infection by *Candida albicans*, an opportunistic fungus that normally resides in the vagina but is inhibited by the bacteria that normally reside in the same environment.

To confirm this diagnosis, a microscope slide of a direct vaginal smear is prepared from the discharge to check for the presence of yeast. A sample of the discharge accompanies this slide to the microbiology lab to determine if there has been an increase in the population of yeast causing vaginitis. After the microbiology lab confirms the diagnosis, the physician prescribes an antifungal drug for Anita to use to eliminate her secondary yeast infection.

- Why was *Candida* not killed by the antibiotics prescribed for the UTI?

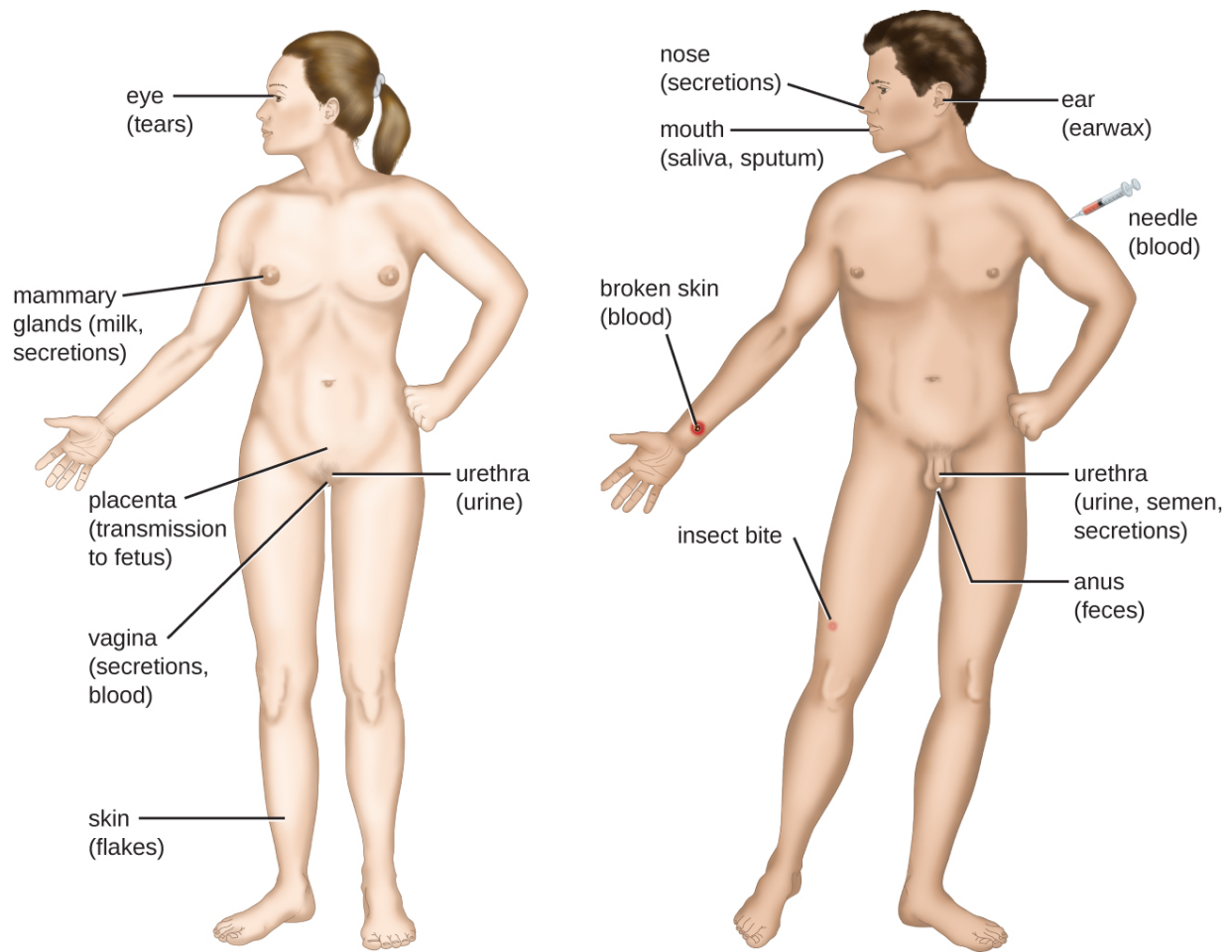
Note:

- List three conditions that could lead to a secondary infection.

Transmission of Disease

For a pathogen to persist, it must put itself in a position to be transmitted to a new host, leaving the infected host through a **portal of exit** ([\[link\]](#)). As with portals of entry, many pathogens are adapted to use a particular portal of exit. Similar to portals of entry, the most common portals of exit include the skin and the respiratory, urogenital, and gastrointestinal tracts.

Coughing and sneezing can expel pathogens from the respiratory tract. A single sneeze can send thousands of virus particles into the air. Secretions and excretions can transport pathogens out of other portals of exit. Feces, urine, semen, vaginal secretions, tears, sweat, and shed skin cells can all serve as vehicles for a pathogen to leave the body. Pathogens that rely on insect vectors for transmission exit the body in the blood extracted by a biting insect. Similarly, some pathogens exit the body in blood extracted by needles.



Pathogens leave the body of an infected host through various portals of exit to infect new hosts.

Key Concepts and Summary

- **Koch's postulates** are used to determine whether a particular microorganism is a pathogen. **Molecular Koch's postulates** are used to determine what genes contribute to a pathogen's ability to cause disease.
- **Virulence**, the degree to which a pathogen can cause disease, can be quantified by calculating either the **ID₅₀** or **LD₅₀** of a pathogen on a given population.

- **Primary pathogens** are capable of causing pathological changes associated with disease in a healthy individual, whereas **opportunistic pathogens** can only cause disease when the individual is compromised by a break in protective barriers or immunosuppression.
- Infections and disease can be caused by pathogens in the environment or microbes in an individual's **resident microbiota**.
- Infections can be classified as **local**, **focal**, or **systemic** depending on the extent to which the pathogen spreads in the body.
- A **secondary infection** can sometimes occur after the host's defenses or normal microbiota are compromised by a **primary infection** or antibiotic treatment.
- Pathogens enter the body through **portals of entry** and leave through **portals of exit**. The stages of pathogenesis include **exposure**, **adhesion**, **invasion**, **infection**, and **transmission**.

Multiple Choice

Exercise:

Problem:

Which of the following is a pathogen that could not be identified by the original Koch's postulates?

- A. *Staphylococcus aureus*
- B. *Pseudomonas aeruginosa*
- C. Human immunodeficiency virus
- D. *Salmonella enterica* serovar Typhimurium

Solution:

C

Exercise:

Problem:

Pathogen A has an ID₅₀ of 50 particles, pathogen B has an ID₅₀ of 1,000 particles, and pathogen C has an ID₅₀ of 1×10^6 particles. Which pathogen is most virulent?

- A. pathogen A
- B. pathogen B
- C. pathogen C

Solution:

A

Exercise:**Problem:**

Which of the following choices lists the steps of pathogenesis in the correct order?

- A. invasion, infection, adhesion, exposure
- B. adhesion, exposure, infection, invasion
- C. exposure, adhesion, invasion, infection
- D. disease, infection, exposure, invasion

Solution:

C

Fill in the Blank**Exercise:**

Problem:

A(n) _____ pathogen causes disease only when conditions are favorable for the microorganism because of transfer to an inappropriate body site or weakened immunity in an individual.

Solution:

opportunistic

Exercise:**Problem:**

The concentration of pathogen needed to kill 50% of an infected group of test animals is the _____.

Solution:

LD₅₀

Exercise:**Problem:**

A(n) _____ infection is a small region of infection from which a pathogen may move to another part of the body to establish a second infection.

Solution:

focal

Exercise:**Problem:**

Cilia, fimbriae, and pili are all examples of structures used by microbes for _____.

Solution:

adhesion

Critical Thinking

Exercise:

Problem:

Diseases that involve biofilm-producing bacteria are of serious concern. They are not as easily treated compared with those involving free-floating (or planktonic) bacteria. Explain three reasons why biofilm formers are more pathogenic.

Exercise:

Problem:

A microbiologist has identified a new gram-negative pathogen that causes liver disease in rats. She suspects that the bacterium's fimbriae are a virulence factor. Describe how molecular Koch's postulates could be used to test this hypothesis.

Exercise:

Problem:

Acupuncture is a form of alternative medicine that is used for pain relief. Explain how acupuncture could facilitate exposure to pathogens.



Virulence Factors of Bacterial and Viral Pathogens

LEARNING OBJECTIVES

- Explain how virulence factors contribute to signs and symptoms of infectious disease
- Differentiate between endotoxins and exotoxins
- Describe and differentiate between various types of exotoxins
- Describe the mechanisms viruses use for adhesion and antigenic variation

In the previous section, we explained that some pathogens are more virulent than others. This is due to the unique **virulence factors** produced by individual pathogens, which determine the extent and severity of disease they may cause. A pathogen's virulence factors are encoded by genes that can be identified using molecular Koch's postulates. When genes encoding virulence factors are inactivated, virulence in the pathogen is diminished. In this section, we examine various types and specific examples of virulence factors and how they contribute to each step of pathogenesis.

Virulence Factors for Adhesion

As discussed in the previous section, the first two steps in pathogenesis are exposure and adhesion. Recall that an adhesin is a protein or glycoprotein found on the surface of a pathogen that attaches to receptors on the host cell. Adhesins are found on bacterial, viral, fungal, and protozoan pathogens. One example of a bacterial adhesin is type 1 fimbrial adhesin, a molecule found on the tips of fimbriae of enterotoxigenic *E. coli* (ETEC). Recall that fimbriae are hairlike protein bristles on the cell surface. Type 1 fimbrial adhesin allows the fimbriae of ETEC cells to attach to the mannose glycans expressed on intestinal epithelial cells. [\[link\]](#) lists common adhesins found in some of the pathogens we have discussed or will be seeing later in this chapter.

Some Bacterial Adhesins and Their Host Attachment Sites			
Pathogen	Disease	Adhesin	Attachment Site
<i>Streptococcus pyogenes</i>	Strep throat	Protein F	Respiratory epithelial cells
<i>Streptococcus mutans</i>	Dental caries	Adhesin P1	Teeth
<i>Neisseria gonorrhoeae</i>	Gonorrhea	Type IV pili	Urethral epithelial cells
Enterotoxigenic <i>E. coli</i> (ETEC)	Traveler's diarrhea	Type 1 fimbriae	Intestinal epithelial cells
<i>Vibrio cholerae</i>	Cholera	N-methylphenylalanine pili	Intestinal epithelial cells

Note:

Part 3

The presence of bacteria in Michael's blood is a sign of infection, since blood is normally sterile. There is no indication that the bacteria entered the blood through an injury. Instead, it appears the portal of entry was the gastrointestinal route. Based on Michael's symptoms, the results of his blood test, and the fact that Michael was the only one in the family to partake of the hot dogs, the physician suspects that Michael is suffering from a case of listeriosis.

Listeria monocytogenes, the facultative intracellular pathogen that causes listeriosis, is a common contaminant in ready-to-eat foods such as lunch meats and dairy products. Once ingested, these bacteria invade intestinal epithelial cells and translocate to the liver, where they grow inside hepatic cells. Listeriosis is fatal in about one in five normal healthy people, and mortality rates are slightly higher in patients with pre-existing conditions that weaken the immune response. A cluster of

virulence genes encoded on a pathogenicity island is responsible for the pathogenicity of *L. monocytogenes*. These genes are regulated by a transcriptional factor known as peptide chain release factor 1 (PrfA). One of the genes regulated by PrfA is *hyl*, which encodes a toxin known as listeriolysin O (LLO), which allows the bacterium to escape vacuoles upon entry into a host cell. A second gene regulated by PrfA is *actA*, which encodes for a surface protein known as actin assembly-inducing protein (ActA). ActA is expressed on the surface of *Listeria* and polymerizes host actin. This enables the bacterium to produce actin tails, move around the cell's cytoplasm, and spread from cell to cell without exiting into the extracellular compartment.

Michael's condition has begun to worsen. He is now experiencing a stiff neck and hemiparesis (weakness of one side of the body). Concerned that the infection is spreading, the physician decides to conduct additional tests to determine what is causing these new symptoms.

- What kind of pathogen causes listeriosis, and what virulence factors contribute to the signs and symptoms Michael is experiencing?
- Is it likely that the infection will spread from Michael's blood? If so, how might this explain his new symptoms?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Bacterial Exoenzymes and Toxins as Virulence Factors

After exposure and adhesion, the next step in pathogenesis is invasion, which can involve enzymes and toxins. Many pathogens achieve invasion by entering the bloodstream, an effective means of dissemination because blood vessels pass close to every cell in the body. The downside of this mechanism of dispersal is that the blood also includes numerous elements of the immune system. Various terms ending in -emia are used to describe the presence of pathogens in the bloodstream. The presence of bacteria in blood is called **bacteremia**. Bacteremia involving pyogens (pus-forming bacteria) is called pyemia. When viruses are found in the blood, it is called **viremia**. The term **toxemia** describes the condition when toxins are found in the blood. If bacteria are both present and multiplying in the blood, this condition is called **septicemia**.

Patients with septicemia are described as **septic**, which can lead to **shock**, a life-threatening decrease in blood pressure (systolic pressure <90 mm Hg) that prevents cells and organs from receiving enough oxygen and nutrients. Some bacteria can cause shock through the release of toxins (virulence factors that can cause tissue

damage) and lead to low blood pressure. Gram-negative bacteria are engulfed by immune system phagocytes, which then release tumor necrosis factor, a molecule involved in inflammation and fever. Tumor necrosis factor binds to blood capillaries to increase their permeability, allowing fluids to pass out of blood vessels and into tissues, causing swelling, or edema ([\[link\]](#)). With high concentrations of tumor necrosis factor, the inflammatory reaction is severe and enough fluid is lost from the circulatory system that blood pressure decreases to dangerously low levels. This can have dire consequences because the heart, lungs, and kidneys rely on normal blood pressure for proper function; thus, multi-organ failure, shock, and death can occur.



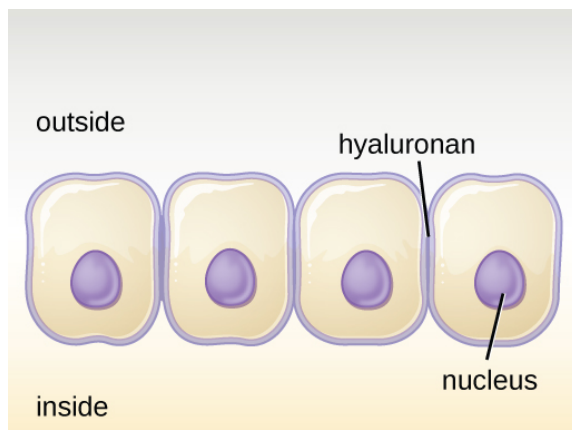
This patient has edema in the tissue of the right hand. Such swelling can occur when bacteria cause the release of pro-inflammatory molecules from immune cells and these molecules cause an increased permeability of blood vessels, allowing fluid to escape the bloodstream and enter tissue.

Exoenzymes

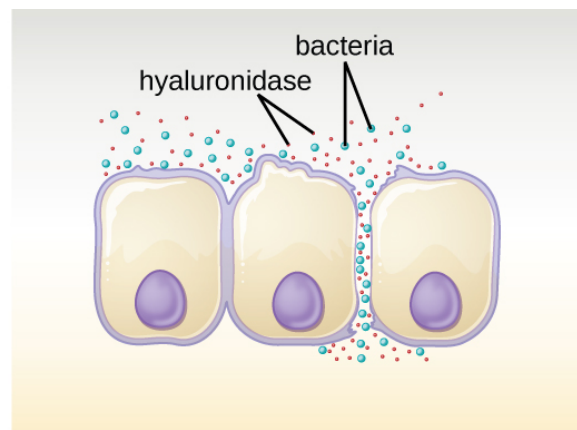
Some pathogens produce extracellular enzymes, or **exoenzymes**, that enable them to invade host cells and deeper tissues. Exoenzymes have a wide variety of targets. Some general classes of exoenzymes and associated pathogens are listed in [\[link\]](#). Each of these exoenzymes functions in the context of a particular tissue structure to facilitate invasion or support its own growth and defend against the immune system. For example, **hyaluronidase S**, an enzyme produced by pathogens like *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Clostridium perfringens*, degrades the glycoside hyaluronan (hyaluronic acid), which acts as an intercellular cement between adjacent cells in connective tissue ([\[link\]](#)). This allows the pathogen to pass through the tissue layers at the portal of entry and disseminate elsewhere in the body ([\[link\]](#)).

Some Classes of Exoenzymes and Their Targets		
Class	Example	Function
Glycohydrolases	Hyaluronidase S in <i>Staphylococcus aureus</i>	Degrades hyaluronic acid that cements cells together to promote spreading through tissues
Nucleases	DNAse produced by <i>S. aureus</i>	Degrades DNA released by dying cells (bacteria and host cells) that can trap the bacteria, thus promoting spread
Phospholipases	Phospholipase C of <i>Bacillus anthracis</i>	Degrades phospholipid bilayer of host cells, causing cellular lysis, and degrade membrane of phagosomes to enable escape into the cytoplasm

Some Classes of Exoenzymes and Their Targets		
Class	Example	Function
Proteases	Collagenase in <i>Clostridium perfringens</i>	Degrades collagen in connective tissue to promote spread



(a)



(b)

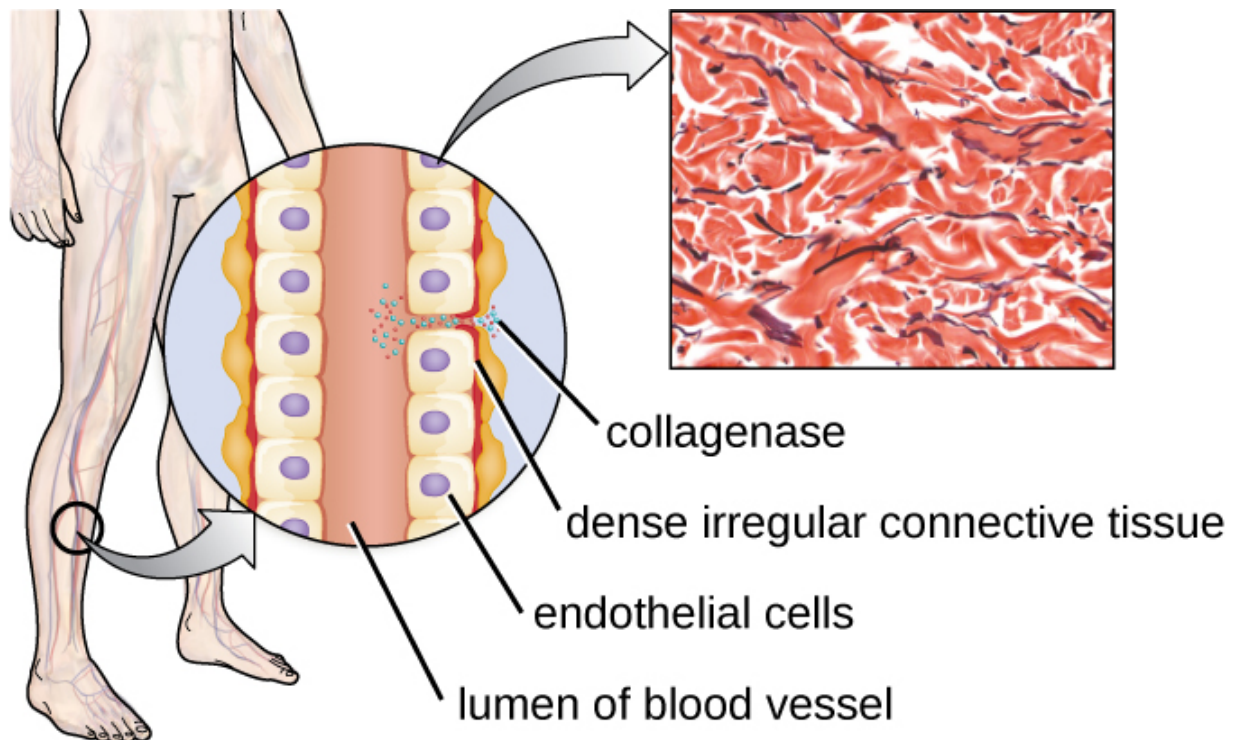
(a) Hyaluronan is a polymer found in the layers of epidermis that connect adjacent cells. (b) Hyaluronidase produced by bacteria degrades this adhesive polymer in the extracellular matrix, allowing passage between cells that would otherwise be blocked.

Pathogen-produced nucleases, such as **DNase** produced by *S. aureus*, degrade extracellular DNA as a means of escape and spreading through tissue. As bacterial and host cells die at the site of infection, they lyse and release their intracellular contents. The DNA chromosome is the largest of the intracellular molecules, and masses of extracellular DNA can trap bacteria and prevent their spread. *S. aureus* produces a DNase to degrade the mesh of extracellular DNA so it can escape and spread to adjacent tissues. This strategy is also used by *S. aureus* and other pathogens to degrade and escape webs of extracellular DNA produced by immune system phagocytes to trap the bacteria.

Enzymes that degrade the phospholipids of cell membranes are called phospholipases. Their actions are specific in regard to the type of phospholipids they act upon and where they enzymatically cleave the molecules. The pathogen responsible for anthrax, *B. anthracis*, produces phospholipase C. When *B. anthracis* is ingested by phagocytic cells of the immune system, phospholipase C degrades the membrane of the phagosome before it can fuse with the lysosome, allowing the pathogen to escape into the cytoplasm and multiply. Phospholipases can also target the membrane that encloses the phagosome within phagocytic cells. As described earlier in this chapter, this is the mechanism used by intracellular pathogens such as *L. monocytogenes* and *Rickettsia* to escape the phagosome and multiply within the cytoplasm of phagocytic cells. The role of phospholipases in bacterial virulence is not restricted to phagosomal escape. Many pathogens produce phospholipases that act to degrade cell membranes and cause lysis of target cells. These phospholipases are involved in lysis of red blood cells, white blood cells, and tissue cells.

Bacterial pathogens also produce various protein-digesting enzymes, or proteases. Proteases can be classified according to their substrate target (e.g., serine proteases target proteins with the amino acid serine) or if they contain metals in their active site (e.g., zinc metalloproteases contain a zinc ion, which is necessary for enzymatic activity).

One example of a protease that contains a metal ion is the exoenzyme **collagenase**. Collagenase digests collagen, the dominant protein in connective tissue. Collagen can be found in the extracellular matrix, especially near mucosal membranes, blood vessels, nerves, and in the layers of the skin. Similar to hyaluronidase, collagenase allows the pathogen to penetrate and spread through the host tissue by digesting this connective tissue protein. The collagenase produced by the gram-positive bacterium *Clostridium perfringens*, for example, allows the bacterium to make its way through the tissue layers and subsequently enter and multiply in the blood (septicemia). *C. perfringens* then uses toxins and a phospholipase to cause cellular lysis and necrosis. Once the host cells have died, the bacterium produces gas by fermenting the muscle carbohydrates. The widespread necrosis of tissue and accompanying gas are characteristic of the condition known as gas gangrene ([link](#)).



The illustration depicts a blood vessel with a single layer of endothelial cells surrounding the lumen and dense connective tissue (shown in red) surrounding the endothelial cell layer. Collagenase produced by *C. perfringens* degrades the collagen between the endothelial cells, allowing the bacteria to enter the bloodstream. (credit illustration: modification of work by Bruce Blaus; credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Note:

Two types of cell death are apoptosis and necrosis. Visit this [website](#) to learn more about the differences between these mechanisms of cell death and their causes.

Toxins

In addition to exoenzymes, certain pathogens are able to produce **toxins**, biological poisons that assist in their ability to invade and cause damage to tissues. The ability of a pathogen to produce toxins to cause damage to host cells is called **toxigenicity**.

Toxins can be categorized as endotoxins or exotoxins. The lipopolysaccharide (LPS) found on the outer membrane of gram-negative bacteria is called **endotoxin** ([\[link\]](#)). During infection and disease, gram-negative bacterial pathogens release endotoxin either when the cell dies, resulting in the disintegration of the membrane, or when the bacterium undergoes binary fission. The lipid component of endotoxin, lipid A, is responsible for the toxic properties of the LPS molecule. Lipid A is relatively conserved across different genera of gram-negative bacteria; therefore, the toxic properties of lipid A are similar regardless of the gram-negative pathogen. In a manner similar to that of tumor necrosis factor, lipid A triggers the immune system's inflammatory response (see [Inflammation and Fever](#)). If the concentration of endotoxin in the body is low, the inflammatory response may provide the host an effective defense against infection; on the other hand, high concentrations of endotoxin in the blood can cause an excessive inflammatory response, leading to a severe drop in blood pressure, multi-organ failure, and death.



Lipopolysaccharide is composed of lipid A, a core glycolipid, and an O-specific polysaccharide side chain. Lipid A is the toxic component that promotes inflammation and fever.

A classic method of detecting endotoxin is by using the *Limulus* amoebocyte lysate (LAL) test. In this procedure, the blood cells (amoebocytes) of the horseshoe crab (*Limulus polyphemus*) is mixed with a patient's serum. The amoebocytes will react to the presence of any endotoxin. This reaction can be observed either chromogenically (color) or by looking for coagulation (clotting reaction) to occur within the serum. An alternative method that has been used is an enzyme-linked immunosorbent assay (ELISA) that uses antibodies to detect the presence of endotoxin.

Unlike the toxic lipid A of endotoxin, **exotoxins** are protein molecules that are produced by a wide variety of living pathogenic bacteria. Although some gram-negative pathogens produce exotoxins, the majority are produced by gram-positive pathogens. Exotoxins differ from endotoxin in several other key characteristics,

summarized in [\[link\]](#). In contrast to endotoxin, which stimulates a general systemic inflammatory response when released, exotoxins are much more specific in their action and the cells they interact with. Each exotoxin targets specific receptors on specific cells and damages those cells through unique molecular mechanisms. Endotoxin remains stable at high temperatures, and requires heating at 121 °C (250 °F) for 45 minutes to inactivate. By contrast, most exotoxins are heat labile because of their protein structure, and many are denatured (inactivated) at temperatures above 41 °C (106 °F). As discussed earlier, endotoxin can stimulate a lethal inflammatory response at very high concentrations and has a measured LD₅₀ of 0.24 mg/kg. By contrast, very small concentrations of exotoxins can be lethal. For example, botulinum toxin, which causes botulism, has an LD₅₀ of 0.000001 mg/kg (240,000 times more lethal than endotoxin).

Comparison of Endotoxin and Exotoxins Produced by Bacteria		
Characteristic	Endotoxin	Exotoxin
Source	Gram-negative bacteria	Gram-positive (primarily) and gram-negative bacteria
Composition	Lipid A component of lipopolysaccharide	Protein
Effect on host	General systemic symptoms of inflammation and fever	Specific damage to cells dependent upon receptor-mediated targeting of cells and specific mechanisms of action
Heat stability	Heat stable	Most are heat labile, but some are heat stable
LD ₅₀	High	Low

The exotoxins can be grouped into three categories based on their target: intracellular targeting, membrane disrupting, and superantigens. [\[link\]](#) provides

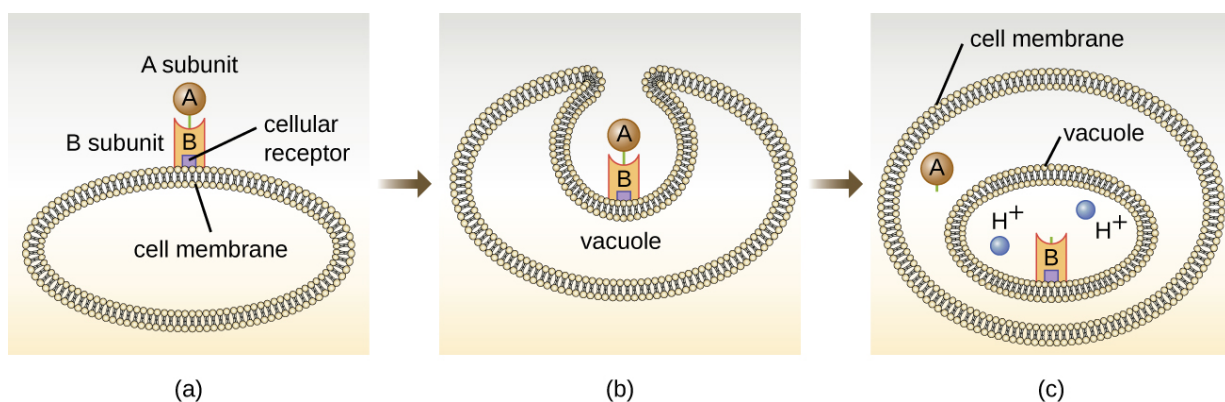
examples of well-characterized toxins within each of these three categories.

Some Common Exotoxins and Associated Bacterial Pathogens			
Category	Example	Pathogen	Mechanism and Disease
Intracellular-targeting toxins	Cholera toxin	<i>Vibrio cholerae</i>	Activation of adenylate cyclase in intestinal cells, causing increased levels of cyclic adenosine monophosphate (cAMP) and secretion of fluids and electrolytes out of cell, causing diarrhea
	Tetanus toxin	<i>Clostridium tetani</i>	Inhibits the release of inhibitory neurotransmitters in the central nervous system, causing spastic paralysis

Some Common Exotoxins and Associated Bacterial Pathogens			
Category	Example	Pathogen	Mechanism and Disease
	Botulinum toxin	<i>Clostridium botulinum</i>	Inhibits release of the neurotransmitter acetylcholine from neurons, resulting in flaccid paralysis
	Diphtheria toxin	<i>Corynebacterium diphtheriae</i>	Inhibition of protein synthesis, causing cellular death
Membrane-disrupting toxins	Streptolysin	<i>Streptococcus pyogenes</i>	Proteins that assemble into pores in cell membranes, disrupting their function and killing the cell
	Pneumolysin	<i>Streptococcus pneumoniae</i>	
	Alpha-toxin	<i>Staphylococcus aureus</i>	
	Alpha-toxin	<i>Clostridium perfringens</i>	Phospholipases that degrade cell membrane phospholipids, disrupting membrane function and killing the cell
	Phospholipase C	<i>Pseudomonas aeruginosa</i>	
	Beta-toxin	<i>Staphylococcus aureus</i>	
Superantigens	Toxic shock syndrome toxin	<i>Staphylococcus aureus</i>	Stimulates excessive activation of immune system cells and release

Some Common Exotoxins and Associated Bacterial Pathogens			
Category	Example	Pathogen	Mechanism and Disease
	Streptococcal mitogenic exotoxin	<i>Streptococcus pyogenes</i>	of cytokines (chemical mediators) from immune system cells. Life-threatening fever, inflammation, and shock are the result.
	Streptococcal pyrogenic toxins	<i>Streptococcus pyogenes</i>	

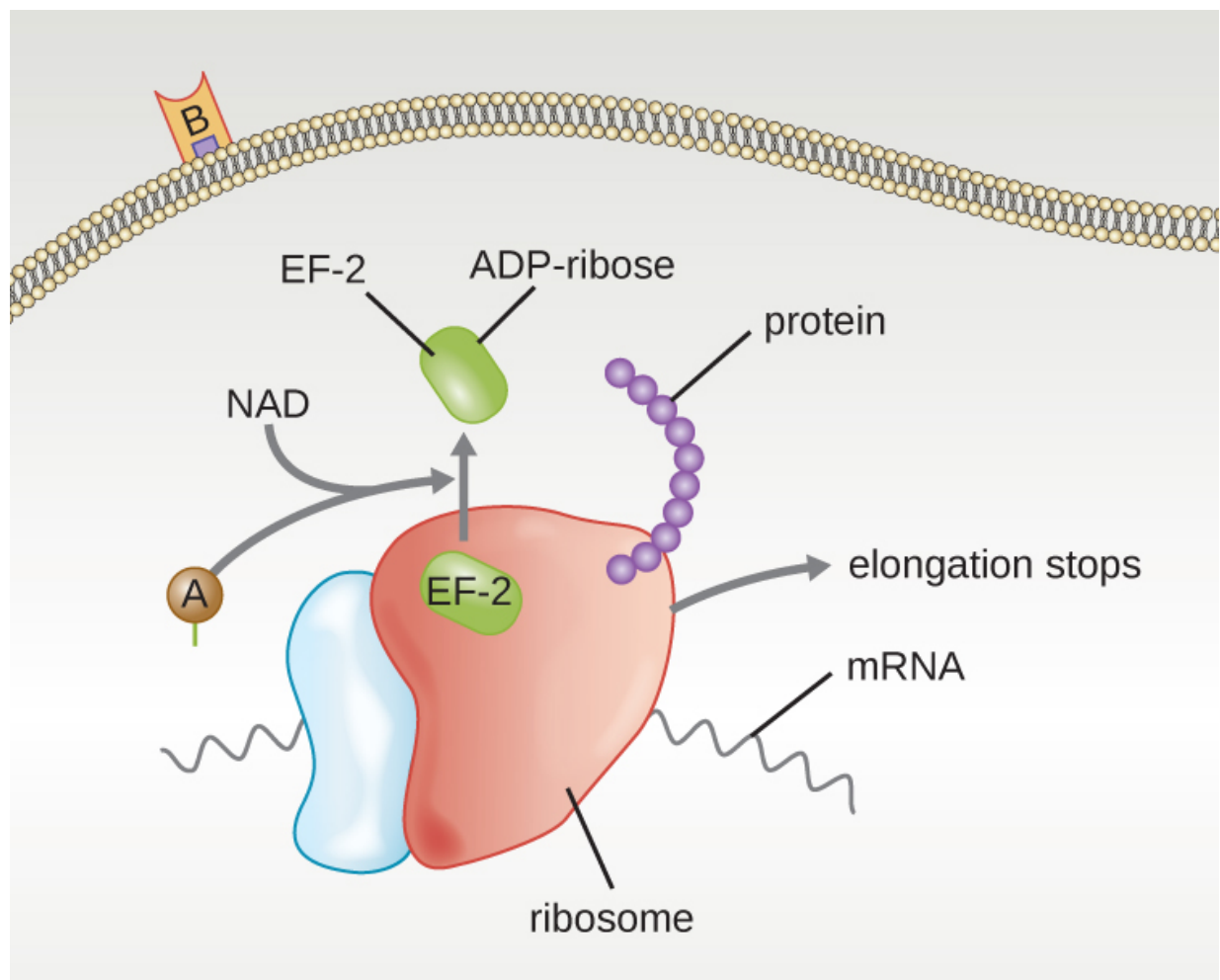
The **intracellular targeting toxins** comprise two components: A for activity and B for binding. Thus, these types of toxins are known as **A-B exotoxins** ([\[link\]](#)). The B component is responsible for the cellular specificity of the toxin and mediates the initial attachment of the toxin to specific cell surface receptors. Once the A-B toxin binds to the host cell, it is brought into the cell by endocytosis and entrapped in a vacuole. The A and B subunits separate as the vacuole acidifies. The A subunit then enters the cell cytoplasm and interferes with the specific internal cellular function that it targets.



- (a) In A-B toxins, the B component binds to the host cell through its interaction with specific cell surface receptors. (b) The toxin is brought in through endocytosis. (c) Once inside the vacuole, the A component (active component) separates from the B component and the A component gains access to the

cytoplasm. (credit: modification of work by “Biology Discussion Forum”/YouTube)

Four unique examples of A-B toxins are the diphtheria, cholera, botulinum, and tetanus toxins. The diphtheria toxin is produced by the gram-positive bacterium *Corynebacterium diphtheriae*, the causative agent of nasopharyngeal and cutaneous diphtheria. After the A subunit of the diphtheria toxin separates and gains access to the cytoplasm, it facilitates the transfer of adenosine diphosphate (ADP)-ribose onto an elongation-factor protein (EF-2) that is needed for protein synthesis. Hence, diphtheria toxin inhibits protein synthesis in the host cell, ultimately killing the cell ([\[link\]](#)).



The mechanism of the diphtheria toxin inhibiting protein synthesis. The A

subunit inactivates elongation factor 2 by transferring an ADP-ribose. This stops protein elongation, inhibiting protein synthesis and killing the cell.

Cholera toxin is an **enterotoxin** produced by the gram-negative bacterium *Vibrio cholerae* and is composed of one A subunit and five B subunits. The mechanism of action of the cholera toxin is complex. The B subunits bind to receptors on the intestinal epithelial cell of the small intestine. After gaining entry into the cytoplasm of the epithelial cell, the A subunit activates an intracellular G protein. The activated G protein, in turn, leads to the activation of the enzyme adenylyl cyclase, which begins to produce an increase in the concentration of cyclic AMP (a secondary messenger molecule). The increased cAMP disrupts the normal physiology of the intestinal epithelial cells and causes them to secrete excessive amounts of fluid and electrolytes into the lumen of the intestinal tract, resulting in severe “rice-water stool” diarrhea characteristic of cholera.

Botulinum toxin (also known as botox) is a neurotoxin produced by the gram-positive bacterium *Clostridium botulinum*. It is the most acutely toxic substance known to date. The toxin is composed of a light A subunit and heavy protein chain B subunit. The B subunit binds to neurons to allow botulinum toxin to enter the neurons at the neuromuscular junction. The A subunit acts as a protease, cleaving proteins involved in the neuron’s release of acetylcholine, a neurotransmitter molecule. Normally, neurons release acetylcholine to induce muscle fiber contractions. The toxin’s ability to block acetylcholine release results in the inhibition of muscle contractions, leading to muscle relaxation. This has the potential to stop breathing and cause death. Because of its action, low concentrations of botox are used for cosmetic and medical procedures, including the removal of wrinkles and treatment of overactive bladder.

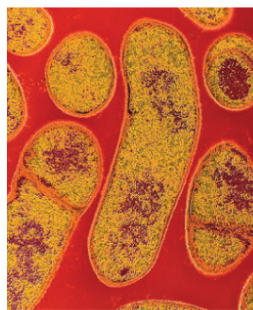
Note:

Click this [link](#) to see an animation of how the cholera toxin functions.

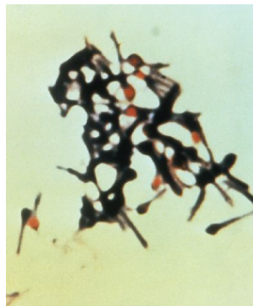
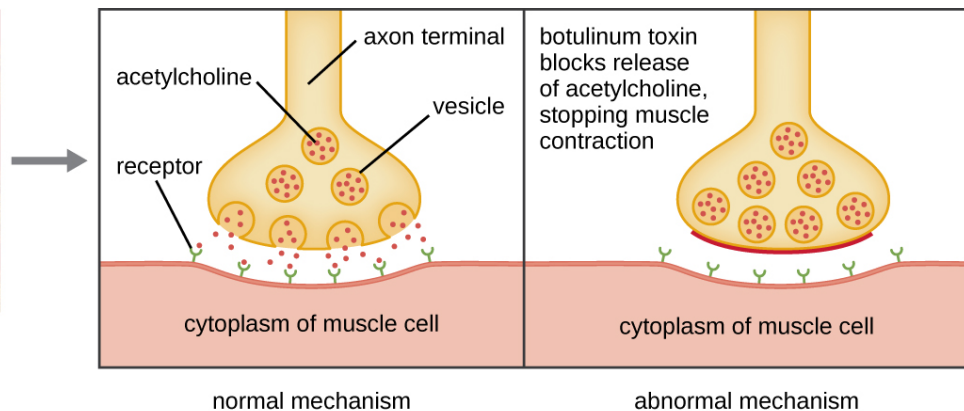
Click this [link](#) to see an animation of how the botulinum toxin functions.

Another neurotoxin is tetanus toxin, which is produced by the gram-positive bacterium *Clostridium tetani*. This toxin also has a light A subunit and heavy protein chain B subunit. Unlike botulinum toxin, tetanus toxin binds to inhibitory interneurons, which are responsible for release of the inhibitory neurotransmitters

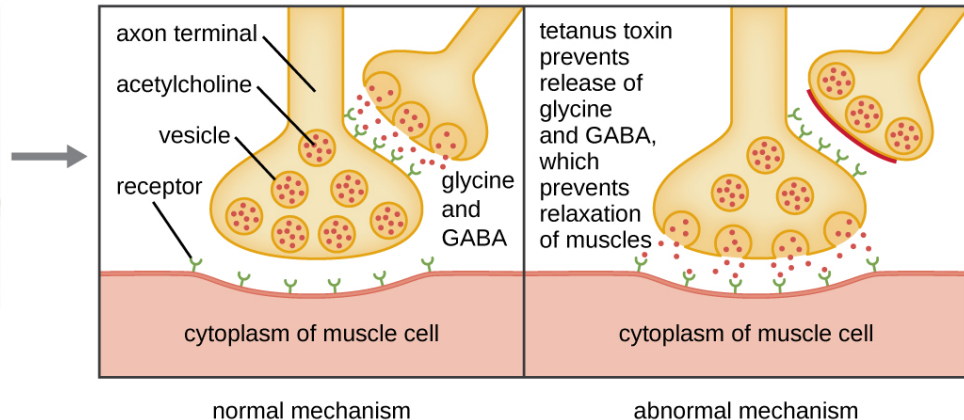
glycine and gamma-aminobutyric acid (GABA). Normally, these neurotransmitters bind to neurons at the neuromuscular junction, resulting in the inhibition of acetylcholine release. Tetanus toxin inhibits the release of glycine and GABA from the interneuron, resulting in permanent muscle contraction. The first symptom is typically stiffness of the jaw (lockjaw). Violent muscle spasms in other parts of the body follow, typically culminating with respiratory failure and death. [\[link\]](#) shows the actions of both botulinum and tetanus toxins.



botulinum toxin
(flaccid paralysis: stops muscle contraction)



tetanus toxin
(spastic paralysis: stops uncontrollable muscle contraction)



Mechanisms of botulinum and tetanus toxins. (credit micrographs: modification of work by Centers for Disease Control and Prevention)

Membrane-disrupting toxins affect cell membrane function either by forming pores or by disrupting the phospholipid bilayer in host cell membranes. Two types of membrane-disrupting exotoxins are **hemolysins** and leukocidins, which form pores in cell membranes, causing leakage of the cytoplasmic contents and cell lysis. These

toxins were originally thought to target red blood cells (erythrocytes) and white blood cells (leukocytes), respectively, but we now know they can affect other cells as well. The gram-positive bacterium *Streptococcus pyogenes* produces streptolysins, water-soluble hemolysins that bind to the cholesterol moieties in the host cell membrane to form a pore. The two types of streptolysins, O and S, are categorized by their ability to cause hemolysis in erythrocytes in the absence or presence of oxygen. Streptolysin O is not active in the presence of oxygen, whereas streptolysin S is active in the presence of oxygen. Other important pore-forming membrane-disrupting toxins include alpha toxin of *Staphylococcus aureus* and pneumolysin of *Streptococcus pneumoniae*.

Bacterial phospholipases are **membrane-disrupting toxins** that degrade the phospholipid bilayer of cell membranes rather than forming pores. We have already discussed the phospholipases associated with *B. anthracis*, *L. pneumophila*, and *Rickettsia* species that enable these bacteria to effect the lysis of phagosomes. These same phospholipases are also hemolysins. Other phospholipases that function as hemolysins include the alpha toxin of *Clostridium perfringens*, phospholipase C of *P. aeruginosa*, and beta toxin of *Staphylococcus aureus*.

Some strains of *S. aureus* also produce a leukocidin called Panton-Valentine leukocidin (PVL). PVL consists of two subunits, S and F. The S component acts like the B subunit of an A-B exotoxin in that it binds to glycolipids on the outer plasma membrane of animal cells. The F-component acts like the A subunit of an A-B exotoxin and carries the enzymatic activity. The toxin inserts and assembles into a pore in the membrane. Genes that encode PVL are more frequently present in *S. aureus* strains that cause skin infections and pneumonia.[\[footnote\]](#) PVL promotes skin infections by causing edema, erythema (reddening of the skin due to blood vessel dilation), and skin necrosis. PVL has also been shown to cause necrotizing pneumonia. PVL promotes pro-inflammatory and cytotoxic effects on alveolar leukocytes. This results in the release of enzymes from the leukocytes, which, in turn, cause damage to lung tissue.

V. Meka. "Panton-Valentine Leukocidin."

<http://www.antimicrobe.org/h04c.files/history/PVL-S-aureus.asp>

The third class of exotoxins is the **superantigens**. These are exotoxins that trigger an excessive, nonspecific stimulation of immune cells to secrete cytokines (chemical messengers). The excessive production of cytokines, often called a cytokine storm, elicits a strong immune and inflammatory response that can cause life-threatening high fevers, low blood pressure, multi-organ failure, shock, and death. The prototype superantigen is the toxic shock syndrome toxin of *S. aureus*. Most toxic shock syndrome cases are associated with vaginal colonization by toxin-producing *S. aureus* in menstruating women; however, colonization of other body sites can also

occur. Some strains of *Streptococcus pyogenes* also produce superantigens; they are referred to as the streptococcal mitogenic exotoxins and the streptococcal pyrogenic toxins.

Note:

- Describe how exoenzymes contribute to bacterial invasion.
- Explain the difference between exotoxins and endotoxin.
- Name the three classes of exotoxins.

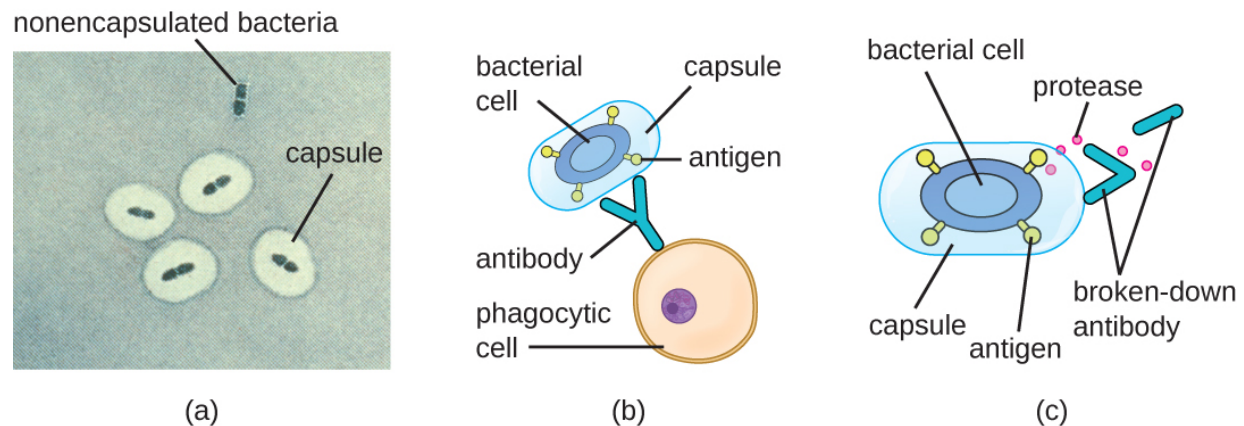
Virulence Factors for Survival in the Host and Immune Evasion

Evading the immune system is also important to invasiveness. Bacteria use a variety of virulence factors to evade phagocytosis by cells of the immune system. For example, many bacteria produce capsules, which are used in adhesion but also aid in immune evasion by preventing ingestion by phagocytes. The composition of the capsule prevents immune cells from being able to adhere and then phagocytose the cell. In addition, the capsule makes the bacterial cell much larger, making it harder for immune cells to engulf the pathogen ([\[link\]](#)). A notable capsule-producing bacterium is the gram-positive pathogen *Streptococcus pneumoniae*, which causes pneumococcal pneumonia, meningitis, septicemia, and other respiratory tract infections. Encapsulated strains of *S. pneumoniae* are more virulent than nonencapsulated strains and are more likely to invade the bloodstream and cause septicemia and meningitis.

Some pathogens can also produce proteases to protect themselves against phagocytosis. As described in [Adaptive Specific Host Defenses](#), the human immune system produces antibodies that bind to surface molecules found on specific bacteria (e.g., capsules, fimbriae, flagella, LPS). This binding initiates phagocytosis and other mechanisms of antibacterial killing and clearance. Proteases combat antibody-mediated killing and clearance by attacking and digesting the antibody molecules ([\[link\]](#)).

In addition to capsules and proteases, some bacterial pathogens produce other virulence factors that allow them to evade the immune system. The fimbriae of certain species of *Streptococcus* contain M protein, which alters the surface of *Streptococcus* and inhibits phagocytosis by blocking the binding of the complement

molecules that assist phagocytes in ingesting bacterial pathogens. The acid-fast bacterium *Mycobacterium tuberculosis* (the causative agent of tuberculosis) produces a waxy substance known as mycolic acid in its cell envelope. When it is engulfed by phagocytes in the lung, the protective mycolic acid coat enables the bacterium to resist some of the killing mechanisms within the phagolysosome.



(a) A micrograph of capsules around bacterial cells. (b) Antibodies normally function by binding to antigens, molecules on the surface of pathogenic bacteria. Phagocytes then bind to the antibody, initiating phagocytosis. (c) Some bacteria also produce proteases, virulence factors that break down host antibodies to evade phagocytosis. (credit a: modification of work by Centers for Disease Control and Prevention)

Some bacteria produce virulence factors that promote infection by exploiting molecules naturally produced by the host. For example, most strains of *Staphylococcus aureus* produce the exoenzyme **coagulase**, which exploits the natural mechanism of blood clotting to evade the immune system. Normally, blood clotting is triggered in response to blood vessel damage; platelets begin to plug the clot, and a cascade of reactions occurs in which fibrinogen, a soluble protein made by the liver, is cleaved into fibrin. Fibrin is an insoluble, thread-like protein that binds to blood platelets, cross-links, and contracts to form a mesh of clumped platelets and red blood cells. The resulting clot prevents further loss of blood from the damaged blood vessels. However, if bacteria release coagulase into the bloodstream, the fibrinogen-to-fibrin cascade is triggered in the absence of blood vessel damage. The resulting clot coats the bacteria in fibrin, protecting the bacteria from exposure to phagocytic immune cells circulating in the bloodstream.

Whereas coagulase causes blood to clot, kinases have the opposite effect by triggering the conversion of plasminogen to plasmin, which is involved in the digestion of fibrin clots. By digesting a clot, kinases allow pathogens trapped in the clot to escape and spread, similar to the way that collagenase, hyaluronidase, and DNase facilitate the spread of infection. Examples of kinases include staphylokinases and streptokinases, produced by *Staphylococcus aureus* and *Streptococcus pyogenes*, respectively. It is intriguing that *S. aureus* can produce both coagulase to promote clotting and staphylokinase to stimulate the digestion of clots. The action of the coagulase provides an important protective barrier from the immune system, but when nutrient supplies are diminished or other conditions signal a need for the pathogen to escape and spread, the production of staphylokinase can initiate this process.

A final mechanism that pathogens can use to protect themselves against the immune system is called **antigenic variation**, which is the alteration of surface proteins so that a pathogen is no longer recognized by the host's immune system. For example, the bacterium *Borrelia burgdorferi*, the causative agent of Lyme disease, contains a surface lipoprotein known as VlsE. Because of genetic recombination during DNA replication and repair, this bacterial protein undergoes antigenic variation. Each time fever occurs, the VlsE protein in *B. burgdorferi* can differ so much that antibodies against previous VlsE sequences are not effective. It is believed that this variation in the VlsE contributes to the ability *B. burgdorferi* to cause chronic disease. Another important human bacterial pathogen that uses antigenic variation to avoid the immune system is *Neisseria gonorrhoeae*, which causes the sexually transmitted disease gonorrhea. This bacterium is well known for its ability to undergo antigenic variation of its type IV pili to avoid immune defenses.

Note:

- Name at least two ways that a capsule provides protection from the immune system.
- Besides capsules, name two other virulence factors used by bacteria to evade the immune system.

Note:

Resolution

Based on Michael's reported symptoms of stiff neck and hemiparesis, the physician suspects that the infection may have spread to his nervous system. The physician decides to order a spinal tap to look for any bacteria that may have invaded the meninges and cerebrospinal fluid (CSF), which would normally be sterile. To perform the spinal tap, Michael's lower back is swabbed with an iodine antiseptic and then covered with a sterile sheet. The needle is aseptically removed from the manufacturer's sealed plastic packaging by the clinician's gloved hands. The needle is inserted and a small volume of fluid is drawn into an attached sample tube. The tube is removed, capped and a prepared label with Michael's data is affixed to it. This STAT (urgent or immediate analysis required) specimen is divided into three separate sterile tubes, each with 1 mL of CSF. These tubes are immediately taken to the hospital's lab, where they are analyzed in the clinical chemistry, hematology, and microbiology departments. The preliminary results from all three departments indicate there is a cerebrospinal infection occurring, with the microbiology department reporting the presence of a gram-positive rod in Michael's CSF. These results confirm what his physician had suspected: Michael's new symptoms are the result of meningitis, acute inflammation of the membranes that protect the brain and spinal cord. Because meningitis can be life threatening and because the first antibiotic therapy was not effective in preventing the spread of infection, Michael is prescribed an aggressive course of two antibiotics, ampicillin and gentamicin, to be delivered intravenously. Michael remains in the hospital for several days for supportive care and for observation. After a week, he is allowed to return home for bed rest and oral antibiotics. After 3 weeks of this treatment, he makes a full recovery.

Go back to the [previous](#) Clinical Focus box.

Viral Virulence

Although viral pathogens are not similar to bacterial pathogens in terms of structure, some of the properties that contribute to their virulence are similar. Viruses use adhesins to facilitate adhesion to host cells, and certain enveloped viruses rely on antigenic variation to avoid the host immune defenses. These virulence factors are discussed in more detail in the following sections.

Viral Adhesins

One of the first steps in any viral infection is adhesion of the virus to specific receptors on the surface of cells. This process is mediated by adhesins that are part

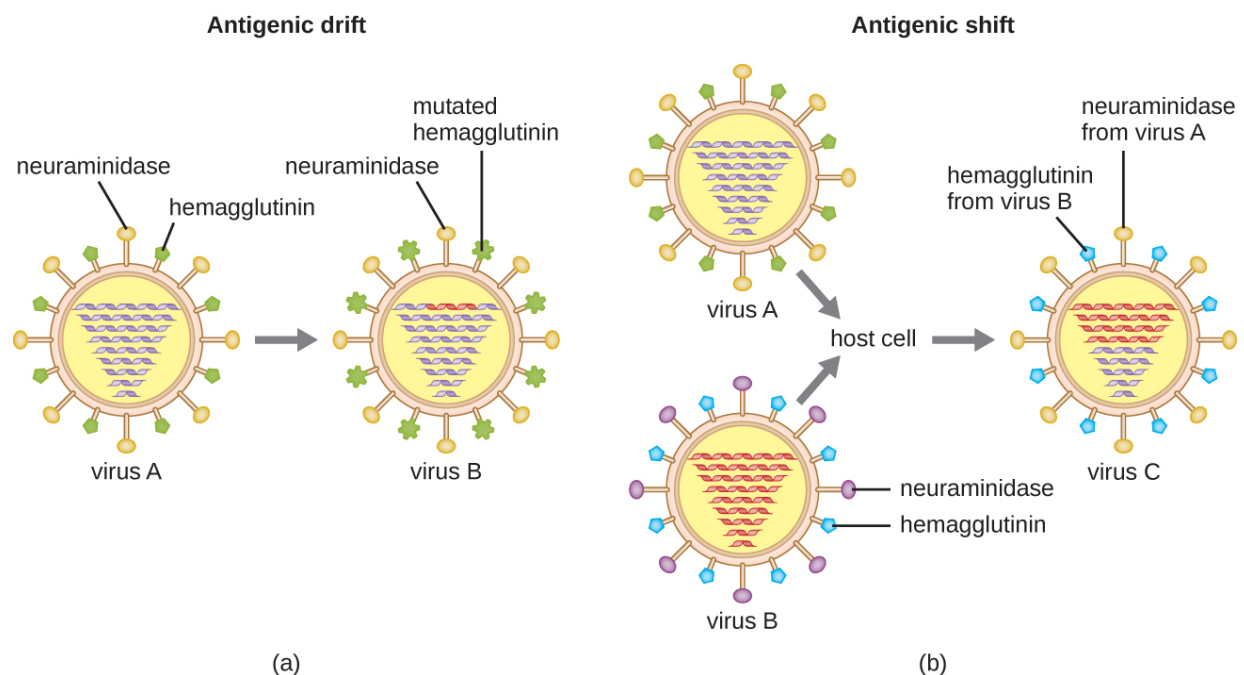
of the viral capsid or membrane envelope. The interaction of viral adhesins with specific cell receptors defines the tropism (preferential targeting) of viruses for specific cells, tissues, and organs in the body. The spike protein hemagglutinin found on Influenzavirus is an example of a viral adhesin; it allows the virus to bind to the sialic acid on the membrane of host respiratory and intestinal cells. Another viral adhesin is the glycoprotein gp20, found on HIV. For HIV to infect cells of the immune system, it must interact with two receptors on the surface of cells. The first interaction involves binding between gp120 and the CD4 cellular marker that is found on some essential immune system cells. However, before viral entry into the cell can occur, a second interaction between gp120 and one of two chemokine receptors (CCR5 and CXCR4) must occur. [\[link\]](#) lists the adhesins for some common viral pathogens and the specific sites to which these adhesins allow viruses to attach.

Some Viral Adhesins and Their Host Attachment Sites			
Pathogen	Disease	Adhesin	Attachment Site
Influenzavirus	Influenza	Hemagglutinin	Sialic acid of respiratory and intestinal cells
Herpes simplex virus I or II	Oral herpes, genital herpes	Glycoproteins gB, gC, gD	Heparan sulfate on mucosal surfaces of the mouth and genitals
Human immunodeficiency virus	HIV/AIDS	Glycoprotein gp120	CD4 and CCR5 or CXCR4 of immune system cells

Antigenic Variation in Viruses

Antigenic variation also occurs in certain types of enveloped viruses, including influenza viruses, which exhibit two forms of antigenic variation: **antigenic drift** and **antigenic shift** ([link](#)). Antigenic drift is the result of point mutations causing slight changes in the spike proteins hemagglutinin (H) and neuraminidase (N). On the other hand, antigenic shift is a major change in spike proteins due to gene reassortment. This reassortment for antigenic shift occurs typically when two different influenza viruses infect the same host.

The rate of antigenic variation in influenza viruses is very high, making it difficult for the immune system to recognize the many different strains of Influenzavirus. Although the body may develop immunity to one strain through natural exposure or vaccination, antigenic variation results in the continual emergence of new strains that the immune system will not recognize. This is the main reason that vaccines against Influenzavirus must be given annually. Each year's influenza vaccine provides protection against the most prevalent strains for that year, but new or different strains may be more prevalent the following year.



Antigenic drift and antigenic shift in influenza viruses. (a) In antigenic drift, mutations in the genes for the surface proteins neuraminidase and/or hemagglutinin result in small antigenic changes over time. (b) In antigenic shift, simultaneous infection of a cell with two different influenza viruses results in mixing of the genes. The resultant virus possesses a mixture of the

proteins of the original viruses. Influenza pandemics can often be traced to antigenic shifts.

Note:

For another explanation of how [antigenic shift and drift](#) occur, watch this video.

Note:

- Describe the role of adhesins in viral tropism.
- Explain the difference between antigenic drift and antigenic shift.

Key Concepts and Summary

- **Virulence factors** contribute to a pathogen's ability to cause disease.
- **Exoenzymes** and **toxins** allow pathogens to invade host tissue and cause tissue damage. Exoenzymes are classified according to the macromolecule they target and exotoxins are classified based on their mechanism of action.
- Bacterial toxins include **endotoxin** and **exotoxins**. Endotoxin is the lipid A component of the LPS of the gram-negative cell envelope. Exotoxins are proteins secreted mainly by gram-positive bacteria, but also are secreted by gram-negative bacteria.
- Bacterial pathogens may evade the host immune response by producing **capsules** to avoid phagocytosis, surviving the intracellular environment of phagocytes, degrading antibodies, or through **antigenic variation**.
- Viral pathogens use adhesins for initiating infections and antigenic variation to avoid immune defenses.
- Influenza viruses use both **antigenic drift** and **antigenic shift** to avoid being recognized by the immune system.

Multiple Choice

Exercise:

Problem: Which of the following would be a virulence factor of a pathogen?

- A. a surface protein allowing the pathogen to bind to host cells
- B. a secondary host the pathogen can infect
- C. a surface protein the host immune system recognizes
- D. the ability to form a provirus

Solution:

A

Exercise:

Problem:

You have recently identified a new toxin. It is produced by a gram-negative bacterium. It is composed mostly of protein, has high toxicity, and is not heat stable. You also discover that it targets liver cells. Based on these characteristics, how would you classify this toxin?

- A. superantigen
- B. endotoxin
- C. exotoxin
- D. leukocidin

Solution:

C

Exercise:

Problem: Which of the following applies to hyaluronidase?

- A. It acts as a spreading factor.
- B. It promotes blood clotting.
- C. It is an example of an adhesin.
- D. It is produced by immune cells to target pathogens.

Solution:

A

Exercise:

Problem: Phospholipases are enzymes that do which of the following?

- A. degrade antibodies
- B. promote pathogen spread through connective tissue.
- C. degrade nucleic acid to promote spread of pathogen
- D. degrade cell membranes to allow pathogens to escape phagosomes

Solution:

D

Fill in the Blank

Exercise:

Problem:

The glycoprotein adhesion gp120 on HIV must interact with _____ on some immune cells as the first step in the process of infecting the cell.

Solution:

CD4

Exercise:

Problem:

Adhesins are usually located on _____ of the pathogen and are composed mainly of _____ and _____.

Solution:

surface; proteins; sugars

Exercise:

Problem: The Shiga and diphtheria toxins target _____ in host cells.

Solution:

protein synthesis

Exercise:**Problem:**

Antigenic _____ is the result of reassortment of genes responsible for the production of influenza virus spike proteins between different virus particles while in the same host, whereas antigenic _____ is the result of point mutations in the spike proteins.

Solution:

shift; drift

Critical Thinking**Exercise:****Problem:**

Two types of toxins are hemolysins and leukocidins. (a) How are these toxins similar? (b) How do they differ?

Exercise:**Problem:**

Imagine that a mutation in the gene encoding the cholera toxin was made. This mutation affects the A-subunit, preventing it from interacting with any host protein. (a) Would the toxin be able to enter into the intestinal epithelial cell? (b) Would the toxin be able to cause diarrhea?

Virulence Factors of Eukaryotic Pathogens

LEARNING OBJECTIVES

- Describe virulence factors unique to fungi and parasites
- Compare virulence factors of fungi and bacteria
- Explain the difference between protozoan parasites and helminths
- Describe how helminths evade the host immune system

Although fungi and parasites are important pathogens causing infectious diseases, their pathogenic mechanisms and virulence factors are not as well characterized as those of bacteria. Despite the relative lack of detailed mechanisms, the stages of pathogenesis and general mechanisms of virulence involved in disease production by these pathogens are similar to those of bacteria.

Fungal Virulence

Pathogenic fungi can produce virulence factors that are similar to the bacterial virulence factors that have been discussed earlier in this chapter. In this section, we will look at the virulence factors associated with species of *Candida*, *Cryptococcus*, *Claviceps*, and *Aspergillus*.

Candida albicans is an opportunistic fungal pathogen and causative agent of oral thrush, vaginal yeast infections, and cutaneous candidiasis. *Candida* produces adhesins (surface glycoproteins) that bind to the phospholipids of epithelial and endothelial cells. To assist in spread and tissue invasion, *Candida* produces proteases and phospholipases (i.e., exoenzymes). One of

these proteases degrades keratin, a structural protein found on epithelial cells, enhancing the ability of the fungus to invade host tissue. In animal studies, it has been shown that the addition of a protease inhibitor led to attenuation of *Candida* infection.[\[footnote\]](#) Similarly, the phospholipases can affect the integrity of host cell membranes to facilitate invasion.

K. Fallon et al. “Role of Aspartic Proteases in Disseminated *Candida albicans* Infection in Mice.” *Infection and Immunity* 65 no. 2 (1997):551–556.

The main virulence factor for *Cryptococcus*, a fungus that causes pneumonia and meningitis, is capsule production. The polysaccharide glucuronoxylomannan is the principal constituent of the *Cryptococcus* capsule. Similar to encapsulated bacterial cells, encapsulated *Cryptococcus* cells are more resistant to phagocytosis than nonencapsulated *Cryptococcus*, which are effectively phagocytosed and, therefore, less virulent.

Like some bacteria, many fungi produce exotoxins. Fungal toxins are called **mycotoxins**. *Claviceps purpurea*, a fungus that grows on rye and related grains, produces a mycotoxin called ergot toxin, an alkaloid responsible for the disease known as ergotism. There are two forms of ergotism: gangrenous and convulsive. In gangrenous ergotism, the ergot toxin causes vasoconstriction, resulting in improper blood flow to the extremities, eventually leading to gangrene. A famous outbreak of gangrenous ergotism occurred in Eastern Europe during the 5th century AD due to the consumption of rye contaminated with *C. purpurea*. In convulsive ergotism, the toxin targets the central nervous system, causing mania and hallucinations.

The mycotoxin aflatoxin is a virulence factor produced by the fungus *Aspergillus*, an opportunistic pathogen that can enter the body via contaminated food or by inhalation. Inhalation of the fungus can lead to the chronic pulmonary disease aspergillosis, characterized by fever, bloody sputum, and/or asthma. Aflatoxin acts in the host as both a mutagen (a substance that causes mutations in DNA) and a **carcinogen** (a substance involved in causing cancer), and has been associated with the development of liver cancer. Aflatoxin has also been shown to cross the blood-placental

barrier.[\[footnote\]](#) A second mycotoxin produced by *Aspergillus* is gliotoxin. This toxin promotes virulence by inducing host cells to self-destruct and by evading the host's immune response by inhibiting the function of phagocytic cells as well as the pro-inflammatory response. Like *Candida*, *Aspergillus* also produces several proteases. One is elastase, which breaks down the protein elastin found in the connective tissue of the lung, leading to the development of lung disease. Another is catalase, an enzyme that protects the fungus from hydrogen peroxide produced by the immune system to destroy pathogens.

C.P. Wild et al. "In-utero exposure to aflatoxin in west Africa." *Lancet* 337 no. 8757 (1991):1602.

Note:

- List virulence factors common to bacteria and fungi.
- What functions do mycotoxins perform to help fungi survive in the host?

Protozoan Virulence

Protozoan pathogens are unicellular eukaryotic parasites that have virulence factors and pathogenic mechanisms analogous to prokaryotic and viral pathogens, including adhesins, toxins, antigenic variation, and the ability to survive inside phagocytic vesicles.

Protozoans often have unique features for attaching to host cells. The protozoan *Giardia lamblia*, which causes the intestinal disease giardiasis, uses a large adhesive disc composed of microtubules to attach to the intestinal mucosa. During adhesion, the flagella of *G. lamblia* move in a manner that draws fluid out from under the disc, resulting in an area of lower pressure that facilitates adhesion to epithelial cells. *Giardia* does not invade the intestinal cells but rather causes inflammation (possibly through

the release of cytopathic substances that cause damage to the cells) and shortens the intestinal villi, inhibiting absorption of nutrients.

Some protozoans are capable of antigenic variation. The obligate intracellular pathogen *Plasmodium falciparum* (one of the causative agents of malaria) resides inside red blood cells, where it produces an adhesin membrane protein known as PfEMP1. This protein is expressed on the surface of the infected erythrocytes, causing blood cells to stick to each other and to the walls of blood vessels. This process impedes blood flow, sometimes leading to organ failure, anemia, jaundice (yellowing of skin and sclera of the eyes due to buildup of bilirubin from lysed red blood cells), and, subsequently, death. Although PfEMP1 can be recognized by the host's immune system, antigenic variations in the structure of the protein over time prevent it from being easily recognized and eliminated. This allows malaria to persist as a chronic infection in many individuals.

The virulence factors of *Trypanosoma brucei*, the causative agent of African sleeping sickness, include the abilities to form capsules and undergo antigenic variation. *T. brucei* evades phagocytosis by producing a dense glycoprotein coat that resembles a bacterial capsule. Over time, host antibodies are produced that recognize this coat, but *T. brucei* is able to alter the structure of the glycoprotein to evade recognition.

Note:

- Explain how antigenic variation by protozoan pathogens helps them survive in the host.

Helminth Virulence

Helminths, or parasitic worms, are multicellular eukaryotic parasites that depend heavily on virulence factors that allow them to gain entry to host tissues. For example, the aquatic larval form of *Schistosoma mansoni*,

which causes schistosomiasis, penetrates intact skin with the aid of proteases that degrade skin proteins, including elastin.

To survive within the host long enough to perpetuate their often-complex life cycles, helminths need to evade the immune system. Some helminths are so large that the immune system is ineffective against them. Others, such as adult roundworms (which cause trichinosis, ascariasis, and other diseases), are protected by a tough outer cuticle.

Over the course of their life cycles, the surface characteristics of the parasites vary, which may help prevent an effective immune response. Some helminths express polysaccharides called glycans on their external surface; because these glycans resemble molecules produced by host cells, the immune system fails to recognize and attack the helminth as a foreign body. This “glycan gimmickry,” as it has been called, serves as a protective cloak that allows the helminth to escape detection by the immune system.

[\[footnote\]](#)

I. van Die, R.D. Cummings. “Glycan Gimmickry by Parasitic Helminths: A Strategy for Modulating the Host Immune Response?” *Glycobiology* 20 no. 1 (2010):2–12.

In addition to evading host defenses, helminths can actively suppress the immune system. *S. mansoni*, for example, degrades host antibodies with proteases. Helminths produce many other substances that suppress elements of both innate nonspecific and adaptive specific host defenses. They also release large amounts of material into the host that may locally overwhelm the immune system or cause it to respond inappropriately.

Note:

- Describe how helminths avoid being destroyed by the host immune system.

Key Concepts and Summary

- Fungal and parasitic pathogens use pathogenic mechanisms and virulence factors that are similar to those of bacterial pathogens
- Fungi initiate infections through the interaction of adhesins with receptors on host cells. Some fungi produce toxins and exoenzymes involved in disease production and capsules that provide protection of phagocytosis.
- Protozoa adhere to target cells through complex mechanisms and can cause cellular damage through release of cytopathic substances. Some protozoa avoid the immune system through antigenic variation and production of capsules.
- Helminthic worms are able to avoid the immune system by coating their exteriors with glycan molecules that make them look like host cells or by suppressing the immune system.

Multiple Choice

Exercise:

Problem:

Which of the following is a major virulence factor for the fungal pathogen *Cryptococcus*?

- A. hemolysin
- B. capsule
- C. collagenase
- D. fimbriae

Solution:

B

Exercise:

Problem:

Which of the following pathogens undergoes antigenic variation to avoid immune defenses?

- A. *Candida*
- B. *Cryptococcus*
- C. *Plasmodium*
- D. *Giardia*

Solution:

C

Fill in the Blank**Exercise:****Problem:**

Candida can invade tissue by producing the exoenzymes _____ and _____.

Solution:

protease and phospholipase

Exercise:**Problem:**

The larval form of *Schistosoma mansoni* uses a _____ to help it gain entry through intact skin.

Solution:

protease

Short Answer

Exercise:

Problem:

Describe the virulence factors associated with the fungal pathogen *Aspergillus*.

Exercise:

Problem: Explain how helminths evade the immune system.

Introduction

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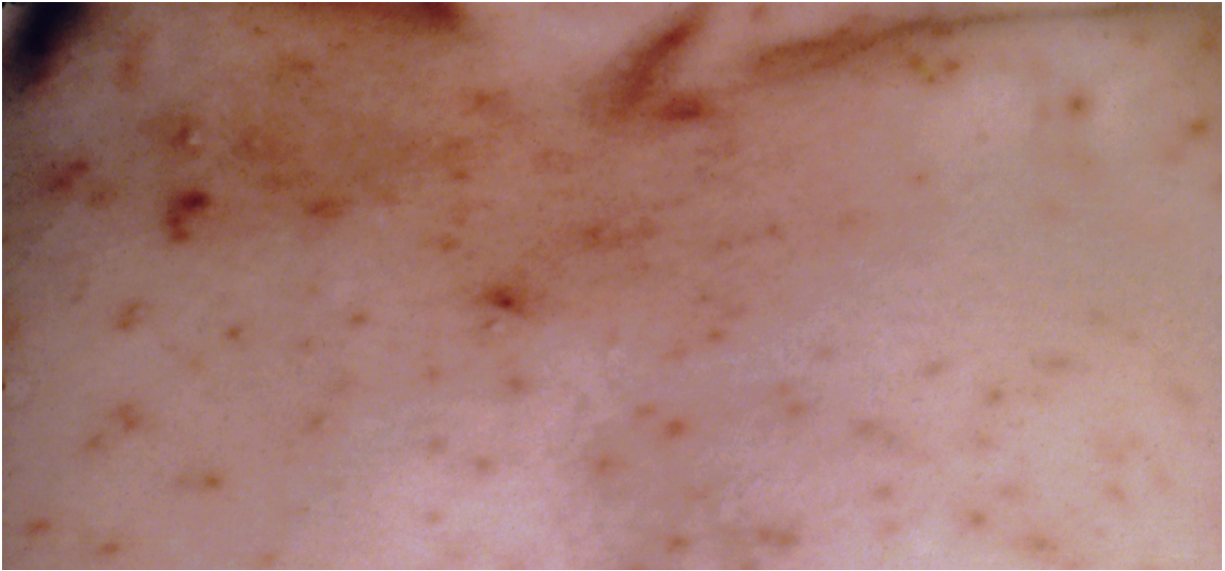
Varicella, or chickenpox, is caused by the highly contagious varicella-zoster virus.

The characteristic rash seen here is partly a result of inflammation associated with the body's immune response to the virus.

Inflammation is a response mechanism of innate immunity that helps the body fight off a wide range of infections.

(credit: modification of work by Centers for

Disease Control and Prevention)



Despite relatively constant exposure to pathogenic microbes in the environment, humans do not generally suffer from constant infection or disease. Under most circumstances, the body is able to defend itself from the threat of infection thanks to a complex immune system designed to repel, kill, and expel disease-causing invaders. Immunity as a whole can be described as two interrelated parts: nonspecific innate immunity, which is the subject of this chapter, and specific adaptive host defenses, which are discussed in the next chapter.

The nonspecific innate immune response provides a first line of defense that can often prevent infections from gaining a solid foothold in the body. These defenses are described as *nonspecific* because they do not target any specific pathogen; rather, they defend against a wide range of potential pathogens. They are called *innate* because they are built-in mechanisms of the human organism. Unlike the specific adaptive defenses, they are not acquired over time and they have no “memory” (they do not improve after repeated exposures to specific pathogens).

Broadly speaking, nonspecific innate defenses provide an immediate (or very rapid) response against potential pathogens. However, these responses are neither perfect nor impenetrable. They can be circumvented by pathogens on occasion, and sometimes they can even cause damage to the body, contributing to the signs and symptoms of infection ([\[link\]](#)).

Inflammation and Fever

LEARNING OBJECTIVES

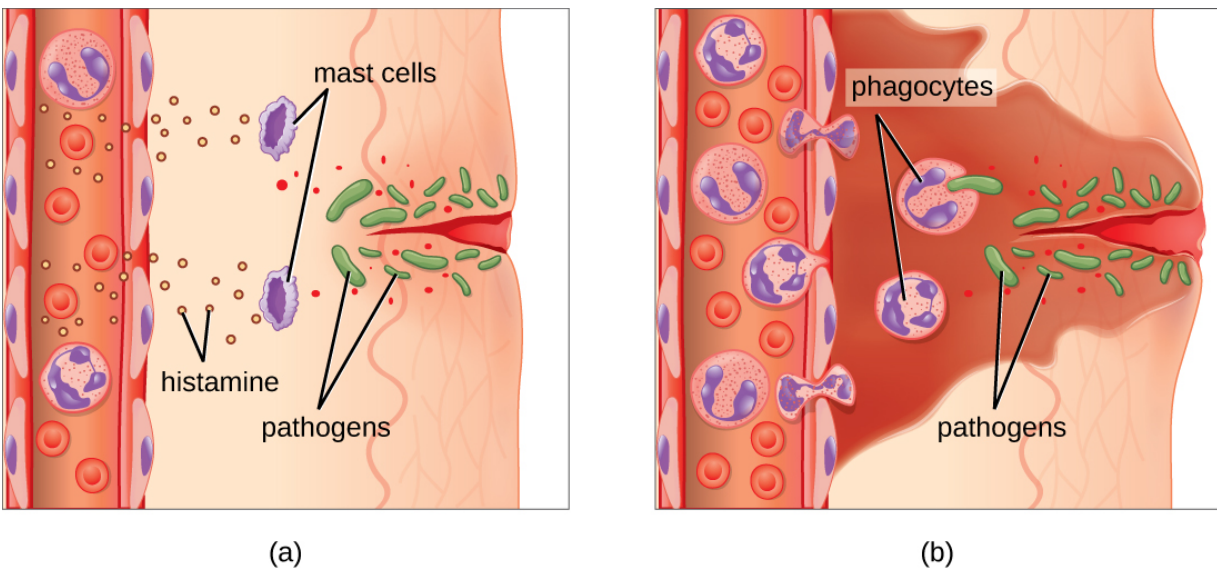
- Identify the signs of inflammation and fever and explain why they occur
- Explain the advantages and risks posed by inflammatory responses

The inflammatory response, or **inflammation**, is triggered by a cascade of chemical mediators and cellular responses that may occur when cells are damaged and stressed or when pathogens successfully breach the physical barriers of the innate immune system. Although inflammation is typically associated with negative consequences of injury or disease, it is a necessary process insofar as it allows for recruitment of the cellular defenses needed to eliminate pathogens, remove damaged and dead cells, and initiate repair mechanisms. Excessive inflammation, however, can result in local tissue damage and, in severe cases, may even become deadly.

Acute Inflammation

An early, if not immediate, response to tissue injury is acute inflammation. Immediately following an injury, vasoconstriction of blood vessels will occur to minimize blood loss. The amount of vasoconstriction is related to the amount of vascular injury, but it is usually brief. Vasoconstriction is followed by vasodilation and increased vascular permeability, as a direct result of the release of histamine from resident mast cells. Increased blood flow and vascular permeability can dilute toxins and bacterial products at the site of injury or infection. They also contribute to the five observable

signs associated with the inflammatory response: **erythema** (redness), **edema** (swelling), heat, pain, and altered function. Vasodilation and increased vascular permeability are also associated with an influx of phagocytes at the site of injury and/or infection. This can enhance the inflammatory response because phagocytes may release proinflammatory chemicals when they are activated by cellular distress signals released from damaged cells, by PAMPs, or by opsonins on the surface of pathogens. Activation of the complement system can further enhance the inflammatory response through the production of the anaphylatoxin C5a. [\[link\]](#) illustrates a typical case of acute inflammation at the site of a skin wound.



(a) Mast cells detect injury to nearby cells and release histamine, initiating an inflammatory response. (b) Histamine increases blood flow to the wound site, and increased vascular permeability allows fluid, proteins, phagocytes, and other immune cells to enter infected tissue. These events result in the swelling and reddening of the injured site, and the increased blood flow to the injured site causes it to feel warm. Inflammation is also associated with pain due to these events stimulating nerve pain receptors in the tissue. The interaction of phagocyte PRRs with cellular distress signals and PAMPs and opsonins on the surface of pathogens leads to the release of more proinflammatory chemicals, enhancing the inflammatory response.

During the period of inflammation, the release of bradykinin causes capillaries to remain dilated, flooding tissues with fluids and leading to edema. Increasing numbers of neutrophils are recruited to the area to fight pathogens. As the fight rages on, pus forms from the accumulation of neutrophils, dead cells, tissue fluids, and lymph. Typically, after a few days, macrophages will help to clear out this pus. Eventually, tissue repair can begin in the wounded area.

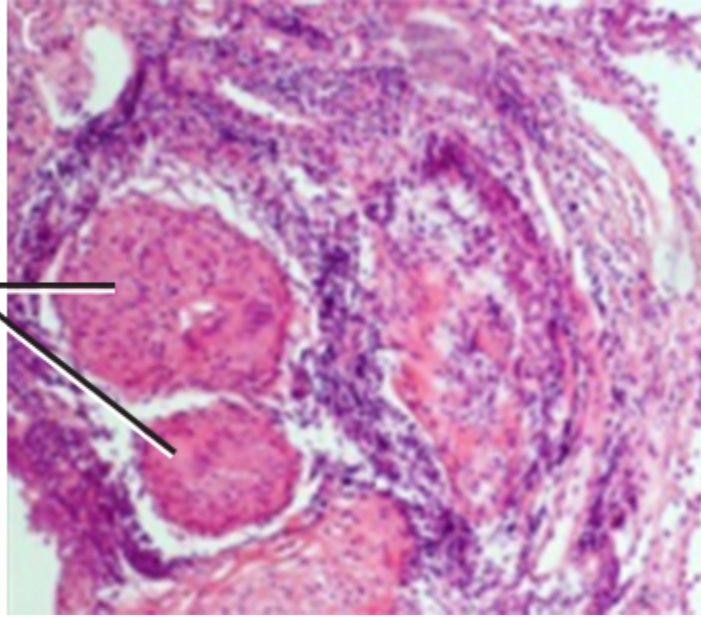
Chronic Inflammation

When acute inflammation is unable to clear an infectious pathogen, chronic inflammation may occur. This often results in an ongoing (and sometimes futile) lower-level battle between the host organism and the pathogen. The wounded area may heal at a superficial level, but pathogens may still be present in deeper tissues, stimulating ongoing inflammation. Additionally, chronic inflammation may be involved in the progression of degenerative neurological diseases such as Alzheimer's and Parkinson's, heart disease, and metastatic cancer.

Chronic inflammation may lead to the formation of **granulomas**, pockets of infected tissue walled off and surrounded by WBCs. Macrophages and other phagocytes wage an unsuccessful battle to eliminate the pathogens and dead cellular materials within a granuloma. One example of a disease that produces chronic inflammation is tuberculosis, which results in the formation of granulomas in lung tissues. A tubercular granuloma is called a tubercle ([\[link\]](#)). Tuberculosis will be covered in more detail in [Bacterial Infections of the Respiratory Tract](#).

Chronic inflammation is not just associated with bacterial infections. Chronic inflammation can be an important cause of tissue damage from viral infections. The extensive scarring observed with hepatitis C infections and liver cirrhosis is the result of chronic inflammation.

granuloma



A tubercle is a granuloma in the lung tissue of a patient with tuberculosis. In this micrograph, white blood cells (stained purple) have walled off a pocket of tissue infected with *Mycobacterium tuberculosis*. Granulomas also occur in many other forms of disease. (credit: modification of work by Piotrowski WJ, Górski P, Duda-Szymańska J, Kwiatkowska S)

Note:

- Name the five signs of inflammation.
- Is a granuloma an acute or chronic form of inflammation? Explain.

Note:

Chronic Edema

In addition to granulomas, chronic inflammation can also result in long-term edema. A condition known as lymphatic filariasis (also known as elephantiasis) provides an extreme example. Lymphatic filariasis is caused by microscopic nematodes (parasitic worms) whose larvae are transmitted between human hosts by mosquitoes. Adult worms live in the lymphatic vessels, where their presence stimulates infiltration by lymphocytes, plasma cells, eosinophils, and thrombocytes (a condition known as lymphangitis). Because of the chronic nature of the illness, granulomas, fibrosis, and blocking of the lymphatic system may eventually occur. Over time, these blockages may worsen with repeated infections over decades, leading to skin thickened with edema and fibrosis. Lymph (extracellular tissue fluid) may spill out of the lymphatic areas and back into tissues, causing extreme swelling ([\[link\]](#)). Secondary bacterial infections commonly follow. Because it is a disease caused by a parasite, eosinophilia (a dramatic rise in the number of eosinophils in the blood) is characteristic of acute infection. However, this increase in antiparasite granulocytes is not sufficient to clear the infection in many cases.

Lymphatic filariasis affects an estimated 120 million people worldwide, mostly concentrated in Africa and Asia.[\[footnote\]](#) Improved sanitation and mosquito control can reduce transmission rates.

Centers for Disease Control and Prevention. “Parasites–Lymphatic Filariasis.” 2016.

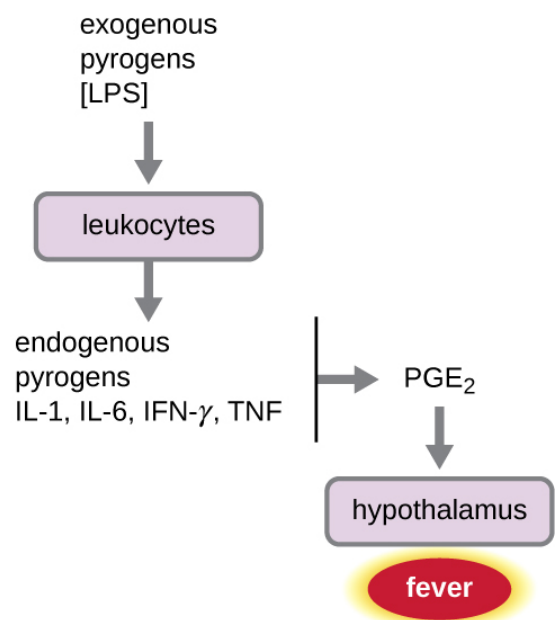
http://www.cdc.gov/parasites/lymphaticfilariasis/gen_info/faqs.html.



Elephantiasis (chronic edema) of the legs due to filariasis. (credit: modification of work by Centers for Disease Control and Prevention)

Fever

A **fever** is an inflammatory response that extends beyond the site of infection and affects the entire body, resulting in an overall increase in body temperature. Body temperature is normally regulated and maintained by the hypothalamus, an anatomical section of the brain that functions to maintain homeostasis in the body. However, certain bacterial or viral infections can result in the production of **pyrogens**, chemicals that effectively alter the “thermostat setting” of the hypothalamus to elevate body temperature and cause fever. Pyrogens may be exogenous or endogenous. For example, the endotoxin lipopolysaccharide (LPS), produced by gram-negative bacteria, is an exogenous pyrogen that may induce the leukocytes to release endogenous pyrogens such as interleukin-1 (IL-1), IL-6, interferon- γ (IFN- γ), and tumor necrosis factor (TNF). In a cascading effect, these molecules can then lead to the release of prostaglandin E2 (PGE₂) from other cells, resetting the hypothalamus to initiate fever ([\[link\]](#)).



The role of the hypothalamus in the inflammatory response. Macrophages recognize pathogens in an area and release cytokines that trigger inflammation. The cytokines also send a signal up the vagus nerve to the hypothalamus.

Like other forms of inflammation, a fever enhances the innate immune defenses by stimulating leukocytes to kill pathogens. The rise in body temperature also may inhibit the growth of many pathogens since human pathogens are mesophiles with optimum growth occurring around 35 °C (95 °F). In addition, some studies suggest that fever may also stimulate release of iron-sequestering compounds from the liver, thereby starving out microbes that rely on iron for growth.[\[footnote\]](#)

N. Parrow et al. “Sequestration and Scavenging of Iron in Infection.” *Infection and Immunity* 81 no. 10 (2013):3503–3514

During fever, the skin may appear pale due to vasoconstriction of the blood vessels in the skin, which is mediated by the hypothalamus to divert blood flow away from extremities, minimizing the loss of heat and raising the core temperature. The hypothalamus will also stimulate shivering of muscles, another effective mechanism of generating heat and raising the core temperature.

The **crisis phase** occurs when the fever breaks. The hypothalamus stimulates vasodilation, resulting in a return of blood flow to the skin and a subsequent release of heat from the body. The hypothalamus also stimulates sweating, which cools the skin as the sweat evaporates.

Although a low-level fever may help an individual overcome an illness, in some instances, this immune response can be too strong, causing tissue and organ damage and, in severe cases, even death. The inflammatory response to bacterial superantigens is one scenario in which a life-threatening fever may develop. Superantigens are bacterial or viral proteins that can cause an excessive activation of T cells from the specific adaptive immune defense, as well as an excessive release of cytokines that overstimulates the inflammatory response. For example, *Staphylococcus aureus* and *Streptococcus pyogenes* are capable of producing superantigens that cause toxic shock syndrome and scarlet fever, respectively. Both of these conditions can be associated with very high, life-threatening fevers in excess of 42 °C (108 °F).

Note:

- Explain the difference between exogenous and endogenous pyrogens.
- How does a fever inhibit pathogens?

Note:**Resolution**

Given her father's premature death, Angela's doctor suspects that she has hereditary angioedema, a genetic disorder that compromises the function of C1 inhibitor protein. Patients with this genetic abnormality may have occasional episodes of swelling in various parts of the body. In Angela's case, the swelling has occurred in the respiratory tract, leading to difficulty breathing. Swelling may also occur in the gastrointestinal tract, causing abdominal cramping, diarrhea, and vomiting, or in the muscles of the face or limbs. This swelling may be nonresponsive to steroid treatment and is often misdiagnosed as an allergy.

Because there are three types of hereditary angioedema, the doctor orders a more specific blood test to look for levels of C1-INH, as well as a functional assay of Angela's C1 inhibitors. The results suggest that Angela has type I hereditary angioedema, which accounts for 80%–85% of all cases. This form of the disorder is caused by a deficiency in C1 esterase inhibitors, the proteins that normally help suppress activation of the complement system. When these proteins are deficient or nonfunctional, overstimulation of the system can lead to production of inflammatory anaphylatoxins, which results in swelling and fluid buildup in tissues. There is no cure for hereditary angioedema, but timely treatment with purified and concentrated C1-INH from blood donors can be effective, preventing tragic outcomes like the one suffered by Angela's father. A number of therapeutic drugs, either currently approved or in late-stage human trials, may also be considered as options for treatment in the near future. These drugs work by inhibiting inflammatory molecules or the receptors for inflammatory molecules.

Thankfully, Angela's condition was quickly diagnosed and treated. Although she may experience additional episodes in the future, her prognosis is good and she can expect to live a relatively normal life provided she seeks treatment at the onset of symptoms.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- **Inflammation** results from the collective response of chemical mediators and cellular defenses to an injury or infection.
- **Acute inflammation** is short lived and localized to the site of injury or infection. **Chronic inflammation** occurs when the inflammatory response is unsuccessful, and may result in the formation of **granulomas** (e.g., with tuberculosis) and scarring (e.g., with hepatitis C viral infections and liver cirrhosis).
- The five cardinal signs of inflammation are **erythema**, **edema**, heat, pain, and altered function. These largely result from innate responses that draw increased blood flow to the injured or infected tissue.
- **Fever** is a system-wide sign of inflammation that raises the body temperature and stimulates the immune response.
- Both inflammation and fever can be harmful if the inflammatory response is too severe.

Multiple Choice

Exercise:

Problem: Which refers to swelling as a result of inflammation?

- A. erythema
- B. edema
- C. granuloma
- D. vasodilation

Solution:

B

Exercise:

Problem:

Which type of inflammation occurs at the site of an injury or infection?

- A. acute
- B. chronic
- C. endogenous
- D. exogenous

Solution:

A

Fill in the Blank**Exercise:****Problem:**

A(n) _____ is a walled-off area of infected tissue that exhibits chronic inflammation.

Solution:

granuloma

Exercise:**Problem:**

The _____ is the part of the body responsible for regulating body temperature.

Solution:

hypothalamus

Exercise:**Problem:**

Heat and redness, or _____, occur when the small blood vessels in an inflamed area dilate (open up), bringing more blood much closer to the surface of the skin.

Solution:

erythema

Short Answer**Exercise:****Problem:**

Differentiate exogenous and endogenous pyrogens, and provide an example of each.

Critical Thinking**Exercise:****Problem:**

If a gram-negative bacterial infection reaches the bloodstream, large quantities of LPS can be released into the blood, resulting in a syndrome called septic shock. Death due to septic shock is a real danger. The overwhelming immune and inflammatory responses that occur with septic shock can cause a perilous drop in blood pressure; intravascular blood clotting; development of thrombi and emboli that block blood vessels, leading to tissue death; failure of multiple organs; and death of the patient. Identify and characterize two to three therapies that might be useful in stopping the dangerous events and outcomes of septic shock once it has begun, given what you have learned about inflammation and innate immunity in this chapter.

Exercise:**Problem:**

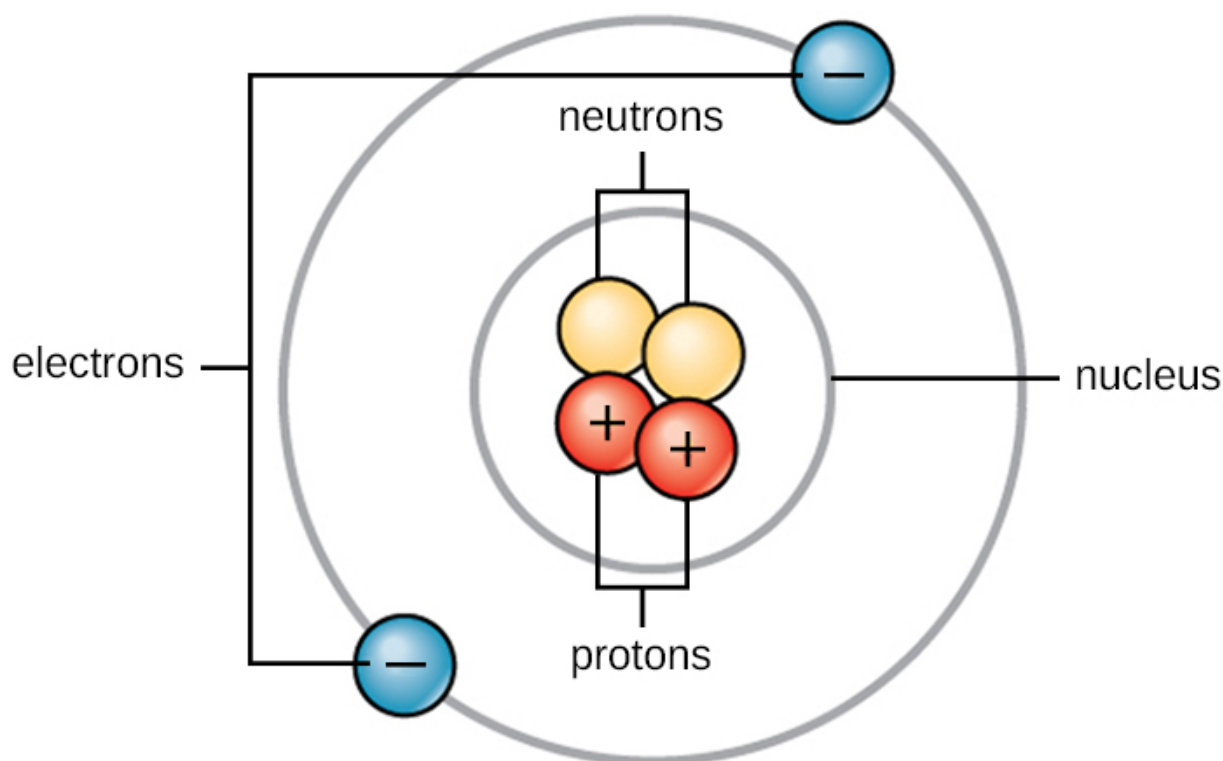
In Lubeck, Germany, in 1930, a group of 251 infants was accidentally administered a tainted vaccine for tuberculosis that contained live *Mycobacterium tuberculosis*. This vaccine was administered orally, directly exposing the infants to the deadly bacterium. Many of these infants contracted tuberculosis, and some died. However, 44 of the infants never contracted tuberculosis. Based on your knowledge of the innate immune system, what innate defenses might have inhibited *M. tuberculosis* enough to prevent these infants from contracting the disease?

Fundamentals of Physics and Chemistry Important to Microbiology

Like all other matter, the matter that comprises microorganisms is governed by the laws of chemistry and physics. The chemical and physical properties of microbial pathogens—both cellular and acellular—dictate their habitat, control their metabolic processes, and determine how they interact with the human body. This appendix provides a review of some of the fundamental principles of chemistry and physics that are essential to an understanding of microbiology. Many of the chapters in this text—especially [Microbial Biochemistry](#) and [Microbial Metabolism](#)—assume that the reader already has an understanding of the concepts reviewed here.

Atomic Structure

Life is made up of matter. Matter occupies space and has mass. All matter is composed of **atoms**. All atoms contain **protons**, **electrons**, and **neutrons** ([\[link\]](#)). The only exception is hydrogen (H), which is made of one proton and one electron. A proton is a positively charged particle that resides in the nucleus (the core of the atom) of an atom and has a mass of 1 atomic mass unit (amu) and a charge of +1. An electron is a negatively charged particle that travels in the space around the nucleus. Electrons are distributed in different energy levels called electron shells. Electrons have a negligible mass and a charge of -1. Neutrons, like protons, reside in the nucleus of an atom. They have a mass of 1 amu and no charge (neutral). The positive (proton) and negative (electron) charges balance each other in a neutral atom, which has a net zero charge. Because protons and neutrons each have a mass of 1 amu, the mass of an atom is equal to the number of protons and neutrons of that atom. The number of electrons does not factor into the overall mass because electron mass is so small.



Atoms are made up of protons and neutrons located within the nucleus and electrons surrounding the nucleus.

Chemical Elements

All matter is composed of atoms of **elements**. Elements have unique physical and chemical properties and are substances that cannot easily be transformed either physically or chemically into other substances. Each element has been given a name, usually derived from Latin or English. The elements also have one- or two-letter symbols representing the name; for example, sodium (Na), gold (Au), and silver (Ag) have abbreviations derived from their original Latin names *natrium*, *aurum*, and *argentum*, respectively. Examples with English abbreviations are carbon (C), hydrogen (H), oxygen (O), and nitrogen (N). A total of 118 different elements (92 of which occur naturally) have been identified and organized into the periodic table of elements. Of the naturally occurring elements, fewer than 30 are found in

organisms on Earth, and four of those (C, H, O, and N) make up approximately 96% of the mass of an organism.[\[footnote\]](#)

Schrijver, Karel, and Iris Schrijver. *Living with the Stars: How the Human Body Is Connected to the Life Cycles of the Earth, the Planets, and the Stars*. Oxford University Press, USA, 2015.

Each unique element is identified by the number of protons in its atomic nucleus. In addition to protons, each element's atomic nucleus contains an equal or greater number of neutrons (with the exception of hydrogen, which has only one proton). The total number of protons per element is described as the **atomic number**, and the combined mass of protons and neutrons is called the atomic mass or **mass number**. Therefore, it is possible to determine the number of neutrons by subtracting the atomic number from the mass number.

Isotopes are different forms of the same element that have the same number of protons, but a different number of neutrons. Many elements have several isotopes with one or two commonly occurring isotopes in nature. For example, carbon-12 (^{12}C), the most common isotope of carbon (98.6% of all C found on Earth),[\[footnote\]](#) contains six protons and six neutrons. Therefore, it has a mass number of 12 (6 protons + 6 neutrons) and an atomic number of 6.

National Oceanic and Atmospheric Administration, "Stable and Radiocarbon Isotopes of Carbon Dioxide." Web page. Accessed Feb 19, 2016 [<http://www.esrl.noaa.gov/gmd/outreach/isotopes/chemistry.html>]

There are two additional types of isotopes in nature: heavy isotopes, and radioisotopes. Heavy isotopes have one or more extra neutrons while still maintaining a stable atomic nucleus. An example of a heavy isotope is carbon-13 (^{13}C) (1.1% of all carbon).[\[footnote\]](#) ^{13}C has a mass number of 13 (6 protons + 7 neutrons). Since the atomic number of ^{13}C is 6, it is still the element carbon; however, it has more mass than the more common form of the element, ^{12}C , because of the extra neutron in the nucleus. Carbon-14 (^{14}C) (0.0001% of all carbon)[\[footnote\]](#) is an example of a radioisotope. ^{14}C has a mass number of 14 (6 protons + 8 neutrons); however, the extra neutrons in ^{14}C result in an unstable nucleus. This instability leads to the process of radioactive decay. Radioactive decay involves the loss of one or

more neutrons and the release of energy in the form of gamma rays, alpha particles, or beta particles (depending on the isotope).

ibid.

ibid.

Heavy isotopes and radioisotopes of carbon and other elements have proven to be useful in research, industry, and medicine.

Chemical Bonds

There are three types of chemical bonds that are important when describing the interaction of atoms both within and between molecules in microbiology: (1) covalent bonds, which can be either polar or non-polar, (2) ionic bonds, and (3) hydrogen bonds. There are other types of interactions such as *London* dispersion forces and *van der Waals* forces that could also be discussed when describing the physical and chemical properties of the intermolecular interactions of atoms, but we will not include descriptions of these forces here.

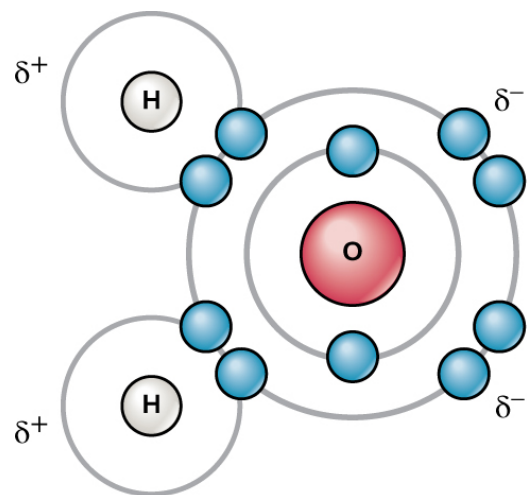
Chemical bonding is determined by the outermost shell of electrons, called the valence electrons (VE), of an atom. The number of VE is important when determining the number and type of chemical bonds an atom will form.

Covalent Bonds

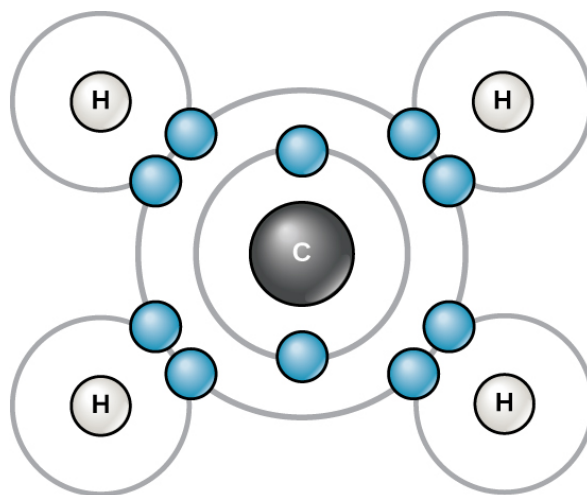
The strongest chemical bond between two or more atoms is a **covalent bond**. These bonds form when an electron is shared between two atoms, and these are the most common form of chemical bond in living organisms. Covalent bonds form between the atoms of elements that make up the biological molecules in our cells. An example of a simple molecule formed with covalent bonds is water, H_2O , with one VE per H atom and 6 VE per O atom. Because of the VE configuration, each H atom is able to accept one additional VE and each O atom is able to accept two additional VE. When sharing electrons, the hydrogen and oxygen atoms that combine to form water molecules become bonded together by covalent bonds ([\[link\]](#)). The electron from the hydrogen atom divides its time between the outer electron

shell of the hydrogen atom and the outermost electron shell of the oxygen atom. To completely fill the outer shell of an oxygen atom, two electrons from two hydrogen atoms are needed, hence the subscript “2” indicating two atoms of H in a molecule of H₂O. This sharing is a lower energy state for all of the atoms involved than if they existed without their outer shells filled.

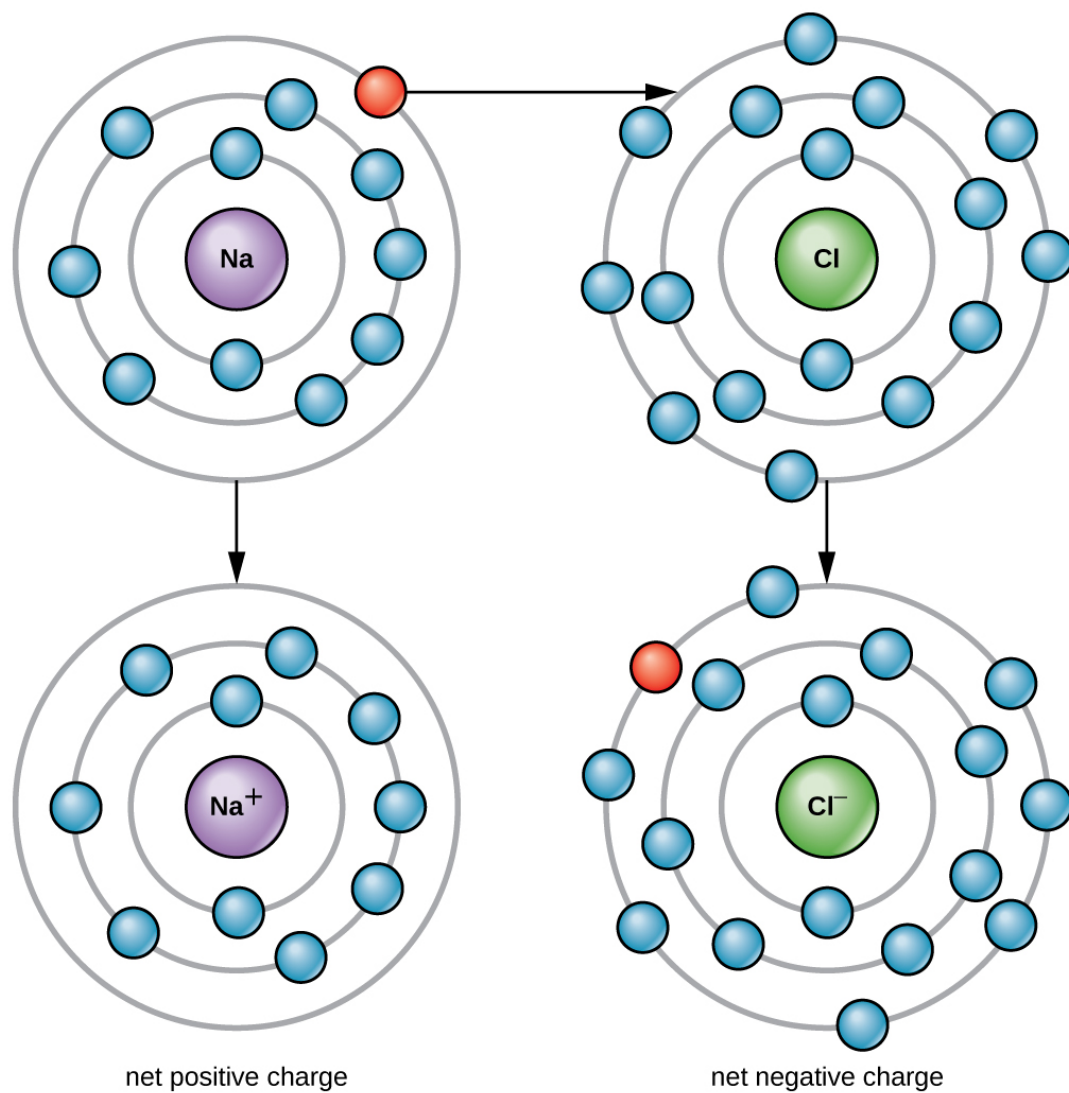
There are two types of covalent bonds: polar and nonpolar. **Nonpolar covalent** bonds form between two atoms of the same or different elements that share the electrons equally ([\[link\]](#)). In a **polar covalent bond**, the electrons shared by the atoms spend more time closer to one nucleus than to the other nucleus. Because of the unequal distribution of electrons between the different nuclei, a slightly positive (δ^+) or slightly negative (δ^-) charge develops. Water is an example of a molecule formed with **polar covalent bonds** ([\[link\]](#)).



Polar covalent bond



Nonpolar covalent bond



Ionic bond

The water molecule (top left) depicts a polar bond with a slightly positive charge on the hydrogen atoms and a slightly negative charge on the oxygen. Methane (top right) is an example of a nonpolar covalent bond. Sodium chloride (bottom) is a substance formed from ionic bonds between sodium and chlorine.

Ions and Ionic Bonds

When an atom does not contain equal numbers of protons and electrons, it is called an **ion**. Because the number of electrons does not equal the number of protons, each ion has a net charge. Positive ions are formed by losing electrons and are called **cations**. Negative ions are formed by gaining electrons and are called **anions**.

For example, a sodium atom has only has one electron in its outermost shell. It takes less energy for the sodium atom to donate that one electron than it does to accept seven more electrons, which it would need to fill its outer shell. If the sodium atom loses an electron, it now has 11 protons and only 10 electrons, leaving it with an overall charge of +1. It is now called a sodium ion (Na^+).

A chlorine atom has seven electrons in its outer shell. Again, it is more energy efficient for the chlorine atom to gain one electron than to lose seven. Therefore, it will more likely gain an electron to form an ion with 17 protons and 18 electrons, giving it a net negative (-1) charge. It is now called a chloride ion (Cl^-). This movement of electrons from one atom to another is referred to as electron transfer. Because positive and negative charges attract, these ions stay together and form an **ionic bond**, or a bond between ions. When Na^+ and Cl^- ions combine to produce NaCl , an electron from a sodium atom stays with the other seven from the chlorine atom, and the sodium and chloride ions attract each other in a lattice of ions with a net zero charge ([link](#)).

Polyatomic ions consist of multiple atoms joined by covalent bonds; but unlike a molecule, a polyatomic ion has a positive or negative charge. It behaves as a cation or anion and can therefore form ionic bonds with other ions to form ionic compounds. The atoms in a polyatomic ion may be from the same element or different elements.

[\[link\]](#) lists some cations and anions that commonly occur in microbiology. Note that this table includes monoatomic as well as polyatomic ions.

Some Common Ions in Microbiology			
Cations		Anions	
sodium	Na^+	chloride	Cl^-
hydrogen	H^+	bicarbonate	HCO_3^-
potassium	K^+	carbonate	CO_3^{2-}
ammonium	NH_4^+	hydrogen sulfate	$\text{H}_2\text{SO}_4^{2-}$
copper (I)	Cu^+	hydrogen sulfide	HS^-
copper (II)	Cu^{2+}	hydroxide	OH^-
iron (II)	Fe^{2+}	hypochlorite	ClO^-
iron (III)	Fe^{3+}	nitrite	NO_2^-
		nitrate	NO_3^-
		peroxide	O_2^{2-}

Some Common Ions in Microbiology			
Cations		Anions	
		phosphate	PO_4^{3-}
		pyrophosphate	$\text{P}_2\text{O}_7^{4-}$
		sulfite	SO_3^{2-}
		thiosulfate	$\text{S}_2\text{O}_3^{2-}$

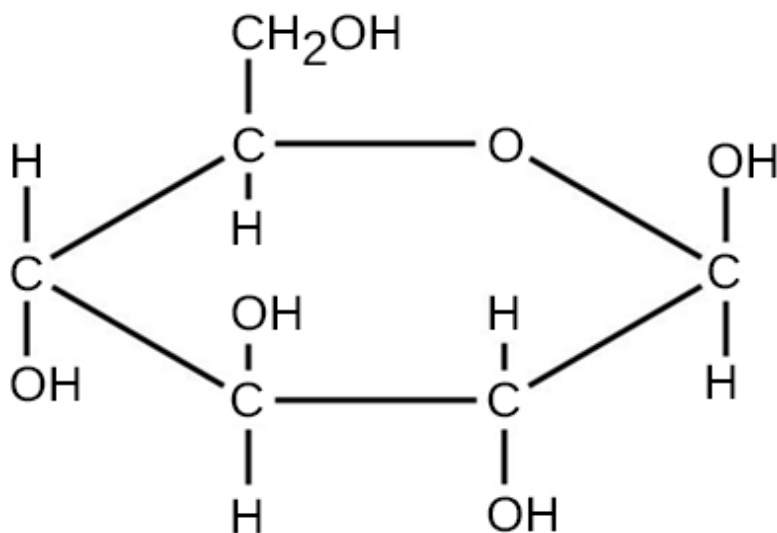
Molecular Formula, Molecular Mass, and the Mole

For molecules formed by covalent bonds, the molecular formula represents the number and types of elemental atoms that compose the molecule. As an example, consider a molecule of glucose, which has the molecular formula $\text{C}_6\text{H}_{12}\text{O}_6$. This molecular formula indicates that a single molecule of glucose is formed from six carbon atoms, twelve hydrogen atoms, and six oxygen atoms.

The **molecular mass** of a molecule can be calculated using the molecular formula and the atomic mass of each element in the molecule. The number of each type of atom is multiplied by the atomic mass; then the products are added to get the molecular mass. For example the molecular mass of glucose, $\text{C}_6\text{H}_{12}\text{O}_6$ ([\[link\]](#)), is calculated as:

Equation:

$$\begin{aligned}
 \text{mass of carbon} &= 12 \frac{\text{amu}}{\text{atom}} \times 6 \text{ atoms} = 72 \text{ amu} \\
 \text{mass of hydrogen} &= 1 \frac{\text{amu}}{\text{atom}} \times 12 \text{ atoms} = 12 \text{ amu} \\
 \text{mass of oxygen} &= 16 \frac{\text{amu}}{\text{atom}} \times 6 \text{ atoms} = 96 \text{ amu} \\
 \text{molecular mass of glucose} &= 72 \text{ amu} + 12 \text{ amu} + 96 \text{ amu} = 180 \text{ amu}
 \end{aligned}$$



The molecular structure of glucose showing the numbers of carbon, oxygen, and hydrogen atoms. Glucose has a molecular mass of 180 amu.

The number of entities composing a mole has been experimentally determined to be 6.022×10^{23} , a fundamental constant named **Avogadro's number** (NA) or the Avogadro constant. This constant is properly reported with an explicit unit of “per mole.”

Energy

Thermodynamics refers to the study of energy and energy transfer involving physical matter.

Matter participating in a particular case of energy transfer is called a system, and everything outside of that matter is called the surroundings. There are two types of systems: open and closed. In an **open system**, energy can be exchanged with its surroundings. A **closed system** cannot exchange energy with its surroundings. Biological organisms are open systems. Energy is exchanged between them and their surroundings as they use energy from the sun to perform photosynthesis or consume energy-storing molecules and

release energy to the environment by doing work and releasing heat. Like all things in the physical world, energy is subject to physical laws. In general, energy is defined as the ability to do work, or to create some kind of change. Energy exists in different forms. For example, electrical energy, light energy, and heat energy are all different types of energy. The **first law of thermodynamics**, often referred to as the law of conservation of energy, states that the total amount of energy in the universe is constant and conserved. Energy exists in many different forms. According to the first law of thermodynamics, energy may be transferred from place to place or transformed into different forms, but it cannot be created or destroyed.

The challenge for all living organisms is to obtain energy from their surroundings in forms that they can transfer or transform into usable energy to do work. Microorganisms have evolved to meet this challenge. Chemical energy stored within organic molecules such as sugars and fats is transferred and transformed through a series of cellular chemical reactions into energy within molecules of ATP. Energy in ATP molecules is easily accessible to do work. Examples of the types of work that cells need to do include building complex molecules, transporting materials, powering the motion of cilia or flagella, and contracting muscle fibers to create movement.

A microorganism's primary tasks of obtaining, transforming, and using energy to do work may seem simple. However, the **second law of thermodynamics** explains why these tasks are more difficult than they appear. All energy transfers and transformations are never completely efficient. In every energy transfer, some amount of energy is lost in a form that is unusable. In most cases, this form is **heat energy**. Thermodynamically, heat energy is defined as the energy transferred from one system to another that is not work. For example, some energy is lost as heat energy during cellular metabolic reactions.

The more energy that is lost by a system to its surroundings, the less ordered and more random the system is. Scientists refer to the measure of randomness or disorder within a system as **entropy**. High entropy means high disorder and low energy. Molecules and chemical reactions have varying entropy as well. For example, entropy increases as molecules at a high concentration in one place diffuse and spread out. The second law of thermodynamics says that energy will always be lost as heat in energy

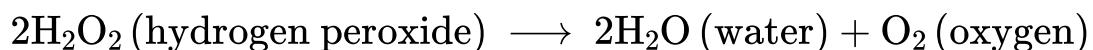
transfers or transformations. Microorganisms are highly ordered, requiring constant energy input to be maintained in a state of low entropy.

Chemical Reactions

Chemical reactions occur when two or more atoms bond together to form molecules or when bonded atoms are broken apart. The substances used in a chemical reaction are called the **reactants** (usually found on the left side of a chemical equation), and the substances produced by the reaction are known as the **products** (usually found on the right side of a chemical equation). An arrow is typically drawn between the reactants and products to indicate the direction of the chemical reaction; this direction is not always a “one-way street.”

An example of a simple chemical reaction is the breaking down of hydrogen peroxide molecules, each of which consists of two hydrogen atoms bonded to two oxygen atoms (H_2O_2). The reactant hydrogen peroxide is broken down into water, containing one oxygen atom bound to two hydrogen atoms (H_2O), and oxygen, which consists of two bonded oxygen atoms (O_2). In the equation below, the reaction includes two hydrogen peroxide molecules and two water molecules. This is an example of a balanced chemical equation, wherein the number of atoms of each element is the same on each side of the equation. According to the law of conservation of matter, the number of atoms before and after a chemical reaction should be equal, such that no atoms are, under normal circumstances, created or destroyed.

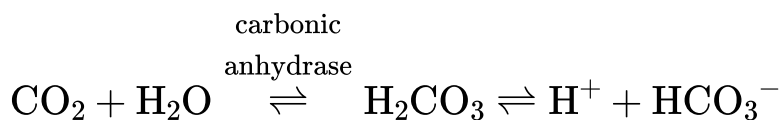
Equation:



Some chemical reactions, such as the one shown above, can proceed in one direction until the reactants are all used up. Equations that describe these reactions contain a unidirectional arrow and are irreversible. **Reversible reactions** are those that can go in either direction. In reversible reactions, reactants are turned into products, but when the concentration of product rises above a certain threshold (characteristic of the particular reaction), some of these products will be converted back into reactants; at this point, the designations of products and reactants are reversed. The changes in

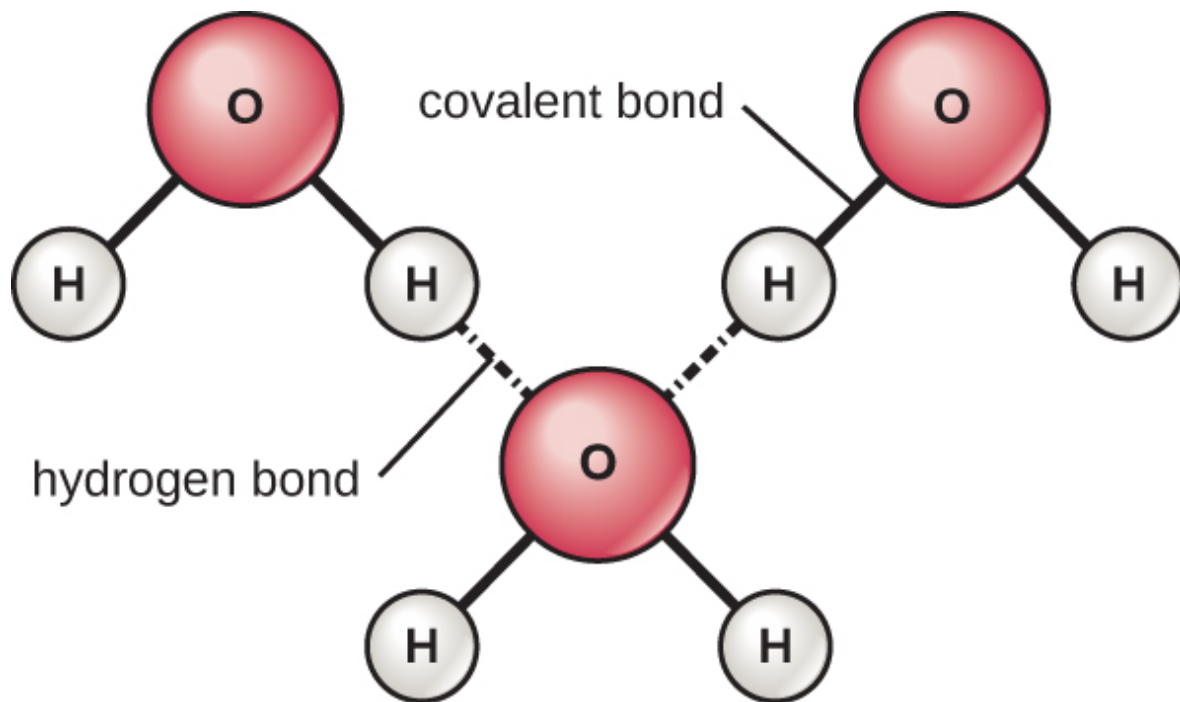
concentration continue until a certain relative balance in concentration between reactants and products occurs—a state called **chemical equilibrium**. At this point, both the forward and reverse reactions continue to occur, but they do so at the same rate, so the concentrations of reactants and products do not change. These situations of reversible reactions are often denoted by a chemical equation with a double-headed arrow pointing towards both the reactants and products. For example, when carbon dioxide dissolves in water, it can do so as a gas dissolved in water *or* by reacting with water to produce carbonic acid. In the cells of some microorganisms, the rate of carbonic acid production is accelerated by the enzyme carbonic anhydrase, as indicated in the following equation:

Equation:



Properties of Water and Solutions

The hydrogen and oxygen atoms within water molecules form polar covalent bonds. There is no overall charge to a water molecule, but there is one δ^+ on each hydrogen atom and two δ^- on the oxygen atom. Each water molecule attracts other water molecules because of the positive and negative charges in the different parts of the molecule ([\[link\]](#)). Water also attracts other polar molecules (such as sugars), forming hydrogen bonds. When a substance readily forms hydrogen bonds with water, it can dissolve in water and is referred to as **hydrophilic** (“water-loving”). Hydrogen bonds are not readily formed with nonpolar substances like oils and fats. These nonpolar compounds are **hydrophobic** (“water-fearing”) and will orient away from and avoid water.



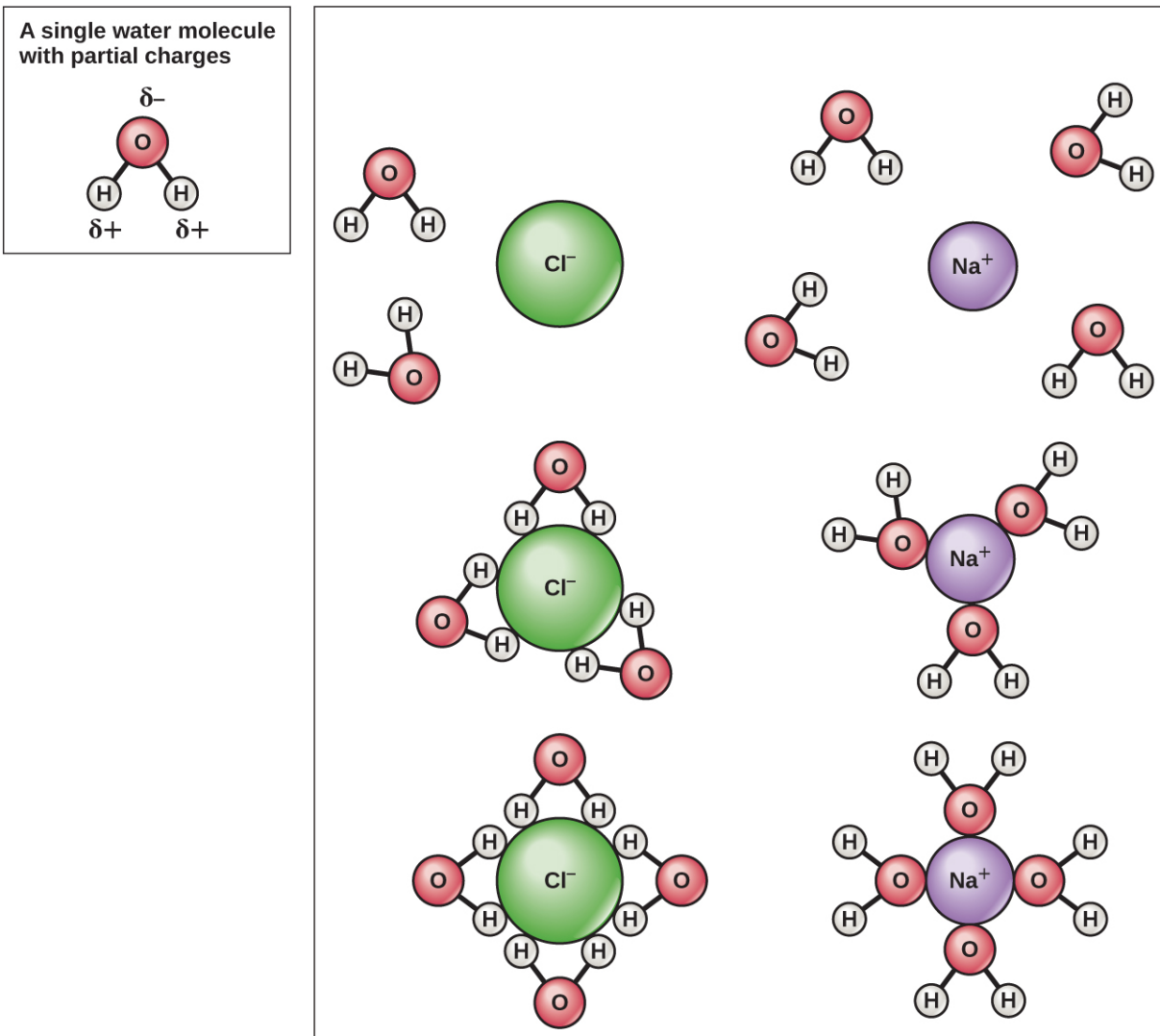
Hydrogen bonds form between slightly positive (δ^+) and slightly negative (δ^-) charges of polar covalent molecules such as water.

The hydrogen bonds in water allow it to absorb and release heat energy more slowly than many other substances. This means that water moderates temperature changes within organisms and in their environments. As energy input continues, the balance between hydrogen-bond formation and breaking swings toward fewer hydrogen bonds: more bonds are broken than are formed. This process results in the release of individual water molecules at the surface of the liquid (such as a body of water, the leaves of a plant, or the skin of an organism) in a process called **evaporation**.

Conversely, as molecular motion decreases and temperatures drop, less energy is present to break the hydrogen bonds between water molecules. These bonds remain intact and begin to form a rigid, lattice-like structure (e.g., ice). When frozen, ice is less dense (the molecules are farther apart) than liquid water. This means that ice floats on the surface of a body of water. In lakes, ponds, and oceans, ice will form on the surface of the water, creating an insulating barrier to protect the animal and plant life beneath

from freezing in the water. If this did not happen, plants and animals living in water would freeze in a block of ice and could not move freely, making life in cold temperatures difficult or impossible.

Because water is polar, with slight positive and negative charges, ionic compounds and polar molecules can readily dissolve in it. Water is, therefore, what is referred to as a solvent—a substance capable of dissolving another substance. The charged particles will form hydrogen bonds with a surrounding layer of water molecules. This is referred to as a **sphere of hydration** and serves to keep the ions separated or dispersed in the water ([\[link\]](#)). These spheres of hydration are also referred to as hydration shells. The polarity of the water molecule makes it an effective solvent and is important in its many roles in living systems.



When table salt (NaCl) is mixed in water, spheres of hydration form around the ions.

The ability of insects to float on and skate across pond water results from the property of **cohesion**. In cohesion, water molecules are attracted to each other (because of hydrogen bonding), keeping the molecules together at the liquid-air (gas) interface. Cohesion gives rise to surface tension, the capacity of a substance to withstand rupture when placed under tension or stress.

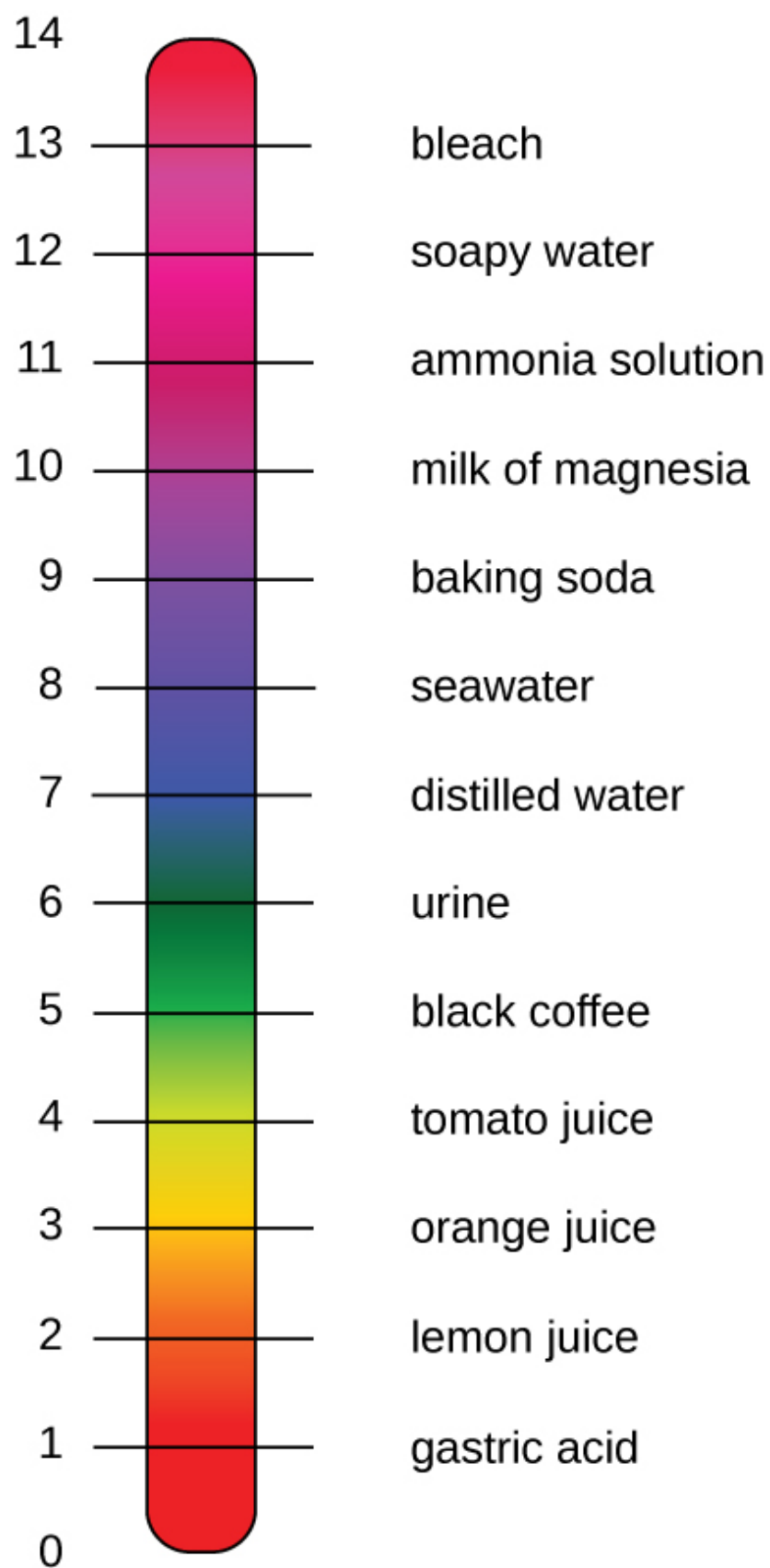
These cohesive forces are also related to water's property of **adhesion**, or the attraction between water molecules and other molecules. This is observed

when water “climbs” up a straw placed in a glass of water. You will notice that the water appears to be higher on the sides of the straw than in the middle. This is because the water molecules are attracted to the straw and therefore adhere to it.

Cohesion and adhesion are also factors in bacterial colonies and biofilm formation. Cohesion keeps the colony intact (helps it “stick” to a surface), while adhesion keeps the cells adhered to each other. Cohesive and adhesive forces are important for sustaining life. For example, because of these forces, water in natural surroundings provides the conditions necessary to allow bacterial and archaeal cells to adhere and accumulate on surfaces.

Acids and Bases

The **pH** of a solution is a measure of hydrogen ion (H^+) and hydroxide ion (OH^-) concentrations and is described as **acidity** or **alkalinity**, respectively. Acidity and alkalinity (also referred to as basicity) can be measured and calculated. pH can be simply represented by the mathematic equation, $pH = -\log_{10}[H^+]$. On the left side of the equation, the "p" means "the negative logarithm of " and the H represents the $[H^+]$. On the right side of the equation, $[H^+]$ is the concentration of H^+ in moles/L . What is not represented in this simple equation is the contribution of the OH^- , which also participates in acidity or alkalinity. Calculation of pH results in a number range of 0 to 14 called the pH scale ([\[link\]](#)). A pH value between 0 and 6.9 indicates an acid. It is also referred to as a low pH, due to a high $[H^+]$ and low $[OH^-]$ concentration. A pH value between 7.1 and 14 indicates an alkali or base. It is also referred to as a high pH, due to a low $[H^+]$ and high $[OH^-]$ concentration. A pH of 7 is described as a neutral pH and occurs when $[H^+]$ equals $[OH^-]$.



The pH scale measures the concentration of hydrogen ions $[H^+]$ and $[OH^-]$ in a substance.

(credit: modification of work by Edward Stevens)

A change of one unit on the pH scale represents a change in the $[H^+]$ by a factor of 10, a change in two units represents a change in the $[H^+]$ by a factor of 100. Thus, small changes in pH represent large changes in $[H^+]$.

Mathematical Basics

Squares and Other Powers

An exponent, or a power, is mathematical shorthand for repeated multiplications. For example, the exponent “2” means to multiply the base for that exponent by itself (in the example here, the base is “5”):

Equation:

$$5^2 = 5 \times 5 = 25$$

The exponent is “2” and the base is the number “5.” This expression (multiplying a number by itself) is also called a square. Any number raised to the power of 2 is being squared. Any number raised to the power of 3 is being cubed:

Equation:

$$5^3 = 5 \times 5 \times 5 = 125$$

A number raised to the fourth power is equal to that number multiplied by itself four times, and so on for higher powers. In general:

Equation:

$$n^x = n \times n^{x-1}$$

Calculating Percents

A percent is a way of expressing a fractional amount of something using a whole divided into 100 parts. A percent is a ratio whose denominator is 100. We use the percent symbol, %, to show percent. Thus, 25% means a ratio of $\frac{25}{100}$, 3% means a ratio of $\frac{3}{100}$, and 100 % percent means $\frac{100}{100}$, or a whole.

Converting Percents

A percent can be converted to a fraction by writing the value of the percent as a fraction with a denominator of 100 and simplifying the fraction if possible.

Equation:

$$25\% = \frac{25}{100} = \frac{1}{4}$$

A percent can be converted to a decimal by writing the value of the percent as a fraction with a denominator of 100 and dividing the numerator by the denominator.

Equation:

$$10\% = \frac{10}{100} = 0.10$$

To convert a decimal to a percent, write the decimal as a fraction. If the denominator of the fraction is not 100, convert it to a fraction with a denominator of 100, and then write the fraction as a percent.

Equation:

$$0.833 = \frac{833}{1000} = \frac{83.3}{100} = 83.3\%$$

To convert a fraction to a percent, first convert the fraction to a decimal, and then convert the decimal to a percent.

Equation:

$$\frac{3}{4} = 0.75 = \frac{75}{100} = 75\%$$

Suppose a researcher finds that 15 out of 23 students in a class are carriers of *Neisseria meningitides*. What percentage of students are carriers? To find

this value, first express the numbers as a fraction.

Equation:

$$\frac{\text{carriers}}{\text{total students}} = \frac{15}{23}$$

Then divide the numerator by the denominator.

Equation:

$$\frac{15}{23} = 15 \div 23 \approx 0.65$$

Finally, to convert a decimal to a percent, multiply by 100.

Equation:

$$0.65 \times 100 = 65\%$$

The percent of students who are carriers is 65%.

You might also get data on occurrence and non-occurrence; for example, in a sample of students, 9 tested positive for *Toxoplasma* antibodies, while 28 tested negative. What is the percentage of seropositive students? The first step is to determine the “whole,” of which the positive students are a part. To do this, sum the positive and negative tests.

Equation:

$$\text{positive} + \text{negative} = 9 + 28 = 37$$

The whole sample consisted of 37 students. The fraction of positives is:

Equation:

$$\frac{\text{positive}}{\text{total students}} = \frac{9}{37}$$

To find the percent of students who are carriers, divide the numerator by the denominator and multiply by 100.

Equation:

$$\frac{9}{37} = 9 \div 37 \approx 0.24$$
$$0.24 \times 100 = 24\%$$

The percent of positive students is about 24%.

Another way to think about calculating a percent is to set up equivalent fractions, one of which is a fraction with 100 as the denominator, and cross-multiply. The previous example would be expressed as:

Equation:

$$\frac{9}{37} = \frac{x}{100}$$

Now, cross multiply and solve for the unknown:

Equation:

9×100	$=$	$37x$	
$\frac{9 \times 100}{37}$	$=$	x	Divide both sides by 37
$\frac{900}{37}$	$=$	x	Multiply
24	$\approx$	x	Divide

The answer, rounded, is the same.

Multiplying and Dividing by Tens

In many fields, especially in the sciences, it is common to multiply decimals by powers of 10. Let's see what happens when we multiply 1.9436 by some powers of 10.

Equation:

$$\begin{aligned}
 1.9436 (10) &= 19.436 \\
 1.9436 (100) &= 194.36 \\
 1.9436 (1000) &= 1943.6
 \end{aligned}$$

The number of places that the decimal point moves is the same as the number of zeros in the power of ten. [\[link\]](#) summarizes the results.

Multiply by	Zeros	Decimal point moves . . .
10	1	1 place to the right
100	2	2 places to the right
1,000	3	3 places to the right
10,000	4	4 places to the right

We can use this pattern as a shortcut to multiply by powers of ten instead of multiplying using the vertical format. We can count the zeros in the power of 10 and then move the decimal point that same number of places to the right.

So, for example, to multiply 45.86 by 100, move the decimal point 2 places to the right.

$$45.86 \times 100 = 4586.$$


Sometimes when we need to move the decimal point, there are not enough decimal places. In that case, we use zeros as placeholders. For example,

let's multiply 2.4 by 100. We need to move the decimal point 2 places to the right. Since there is only one digit to the right of the decimal point, we must write a 0 in the hundredths place.

$$2.4 \times 100 = 240.$$


When dividing by powers of 10, simply take the opposite approach and move the decimal to the left by the number of zeros in the power of ten.

Let's see what happens when we divide 1.9436 by some powers of 10.

Equation:

$$\begin{aligned} 1.9436 \div 10 &= 0.19436 \\ 1.9436 \div 100 &= 0.019436 \\ 1.9436 \div 1000 &= 0.0019436 \end{aligned}$$

If there are insufficient digits to move the decimal, add zeroes to create places.

Scientific Notation

Scientific notation is used to express very large and very small numbers as a product of two numbers. The first number of the product, the digit term, is usually a number not less than 1 and not greater than 10. The second number of the product, the exponential term, is written as 10 with an exponent. Some examples of scientific notation are given in [\[link\]](#).

Standard Notation	Scientific Notation
-------------------	---------------------

Standard Notation	Scientific Notation
1000	1×10^3
100	1×10^2
10	1×10^1
1	1×10^0
0.1	1×10^{-1}
0.01	1×10^{-2}

Scientific notation is particularly useful notation for very large and very small numbers, such as $1,230,000,000 = 1.23 \times 10^9$, and $0.00000000036 = 3.6 \times 10^{-10}$.

Expressing Numbers in Scientific Notation

Converting any number to scientific notation is straightforward. Count the number of places needed to move the decimal next to the left-most non-zero digit: that is, to make the number between 1 and 10. Then multiply that number by 10 raised to the number of places you moved the decimal. The exponent is positive if you moved the decimal to the left and negative if you moved the decimal to the right. So

Equation:

$$2386 = 2.386 \times 1000 = 2.386 \times 10^3$$

and

Equation:

$$0.123 = 1.23 \times 0.1 = 1.23 \times 10^{-1}$$

The power (exponent) of 10 is equal to the number of places the decimal is shifted.

Logarithms

The common logarithm (log) of a number is the power to which 10 must be raised to equal that number. For example, the common logarithm of 100 is 2, because 10 must be raised to the second power to equal 100. Additional examples are in [\[link\]](#).

Number	Exponential Form	Common Logarithm
1000	10^3	3
10	10^1	1
1	10^0	0
0.1	10^{-1}	-1
0.001	10^{-3}	-3

To find the common logarithm of most numbers, you will need to use the LOG button on a calculator.

Rounding and Significant Digits

In reporting numerical data obtained via measurements, we use only as many significant figures as the accuracy of the measurement warrants. For example, suppose a microbiologist using an automated cell counter determines that there are 525,341 bacterial cells in a one-liter sample of

river water. However, she records the concentration as 525,000 cells per liter and uses this rounded number to estimate the number of cells that would likely be found in 10 liters of river water. In this instance, the last three digits of the measured quantity are not considered *significant*. They are rounded to account for variations in the number of cells that would likely occur if more samples were measured.

The importance of significant figures lies in their application to fundamental computation. In addition and subtraction, the sum or difference should contain as many digits to the right of the decimal as that in the *least* certain (indicated by underscoring in the following example) of the numbers used in the computation.

Suppose a microbiologist wishes to calculate the total mass of two samples of agar.

Equation:

$$\begin{array}{r} 4.38\textbf{\underline{3}} \text{ g} \\ 3.002\textbf{\underline{1}} \text{ g} \\ \hline 7.38\textbf{\underline{5}} \text{ g} \end{array}$$

The least certain of the two masses has three decimal places, so the sum must have three decimal places.

In multiplication and division, the product or quotient should contain no more digits than in the factor containing the *least* number of significant figures. Suppose the microbiologist would like to calculate how much of a reagent would be present in 6.6 mL if the concentration is 0.638 g/mL.

Equation:

$$0.63\textbf{\underline{8}} \frac{\text{g}}{\text{mL}} \times 6.\textbf{\underline{6}} \text{ mL} = 4.1 \text{ g}$$

Again, the answer has only one decimal place because this is the accuracy of the least accurate number in the calculation.

When rounding numbers, increase the retained digit by 1 if it is followed by a number larger than 5 (“round up”). Do not change the retained digit if the digits that follow are less than 5 (“round down”). If the retained digit is followed by 5, round up if the retained digit is odd, or round down if it is even (after rounding, the retained digit will thus always be even).

Generation Time

It is possible to write an equation to calculate the cell numbers at any time if the number of starting cells and doubling time are known, as long as the cells are dividing at a constant rate. We define N_0 as the starting number of bacteria, the number at time $t = 0$. N_i is the number of bacteria at time $t = i$, an arbitrary time in the future. Finally we will set j equal to the number of generations, or the number of times the cell population doubles during the time interval. Then we have,

Equation:

$$N_i = N_0 \times 2^j$$

This equation is an expression of growth by binary fission.

In our example, $N_0 = 4$, the number of generations, j , is equal to 3 after 90 minutes because the generation time is 30 minutes. The number of cells can be estimated from the following equation:

Equation:

$$\begin{aligned} N_i &= N_0 \times 2^j \\ N_{90} &= 4 \times 2^3 \\ N_{90} &= 4 \times 8 = 32 \end{aligned}$$

The number of cells after 90 minutes is 32.

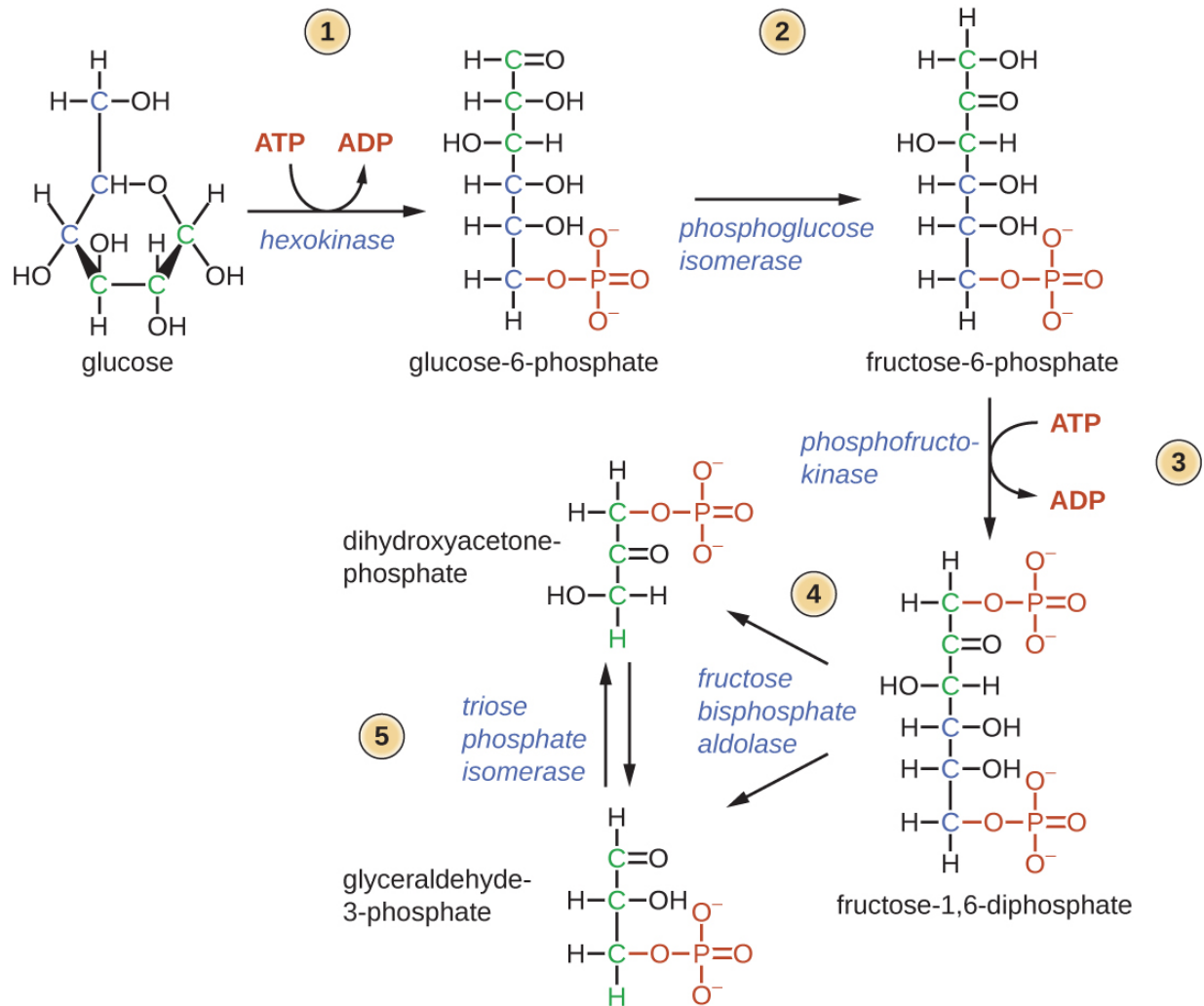
Most Probable Number

The table in [\[link\]](#) contains values used to calculate the most probable number example given in [How Microbes Grow](#).

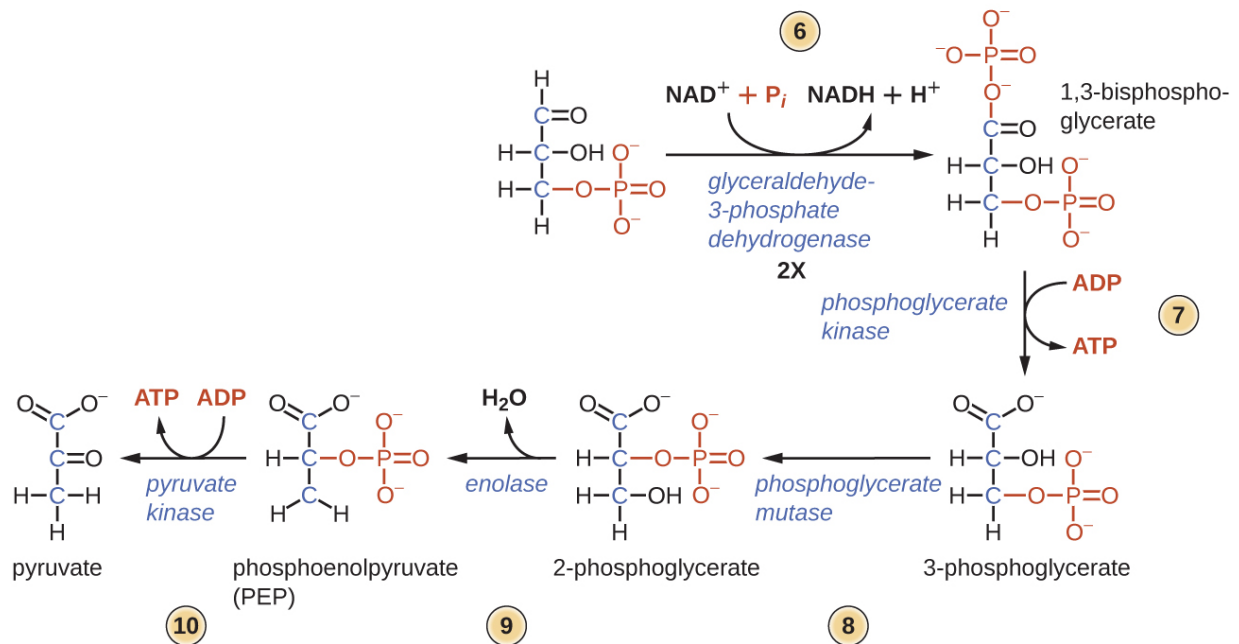
Most Probable Number Table					
Number of tubes giving a positive reaction for a 5-tube set			MPN (per 100 ml)	95% Confidence Limits	
10 ml	1 ml	0.1 ml		Low	High
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500

Metabolic Pathways

Glycolysis

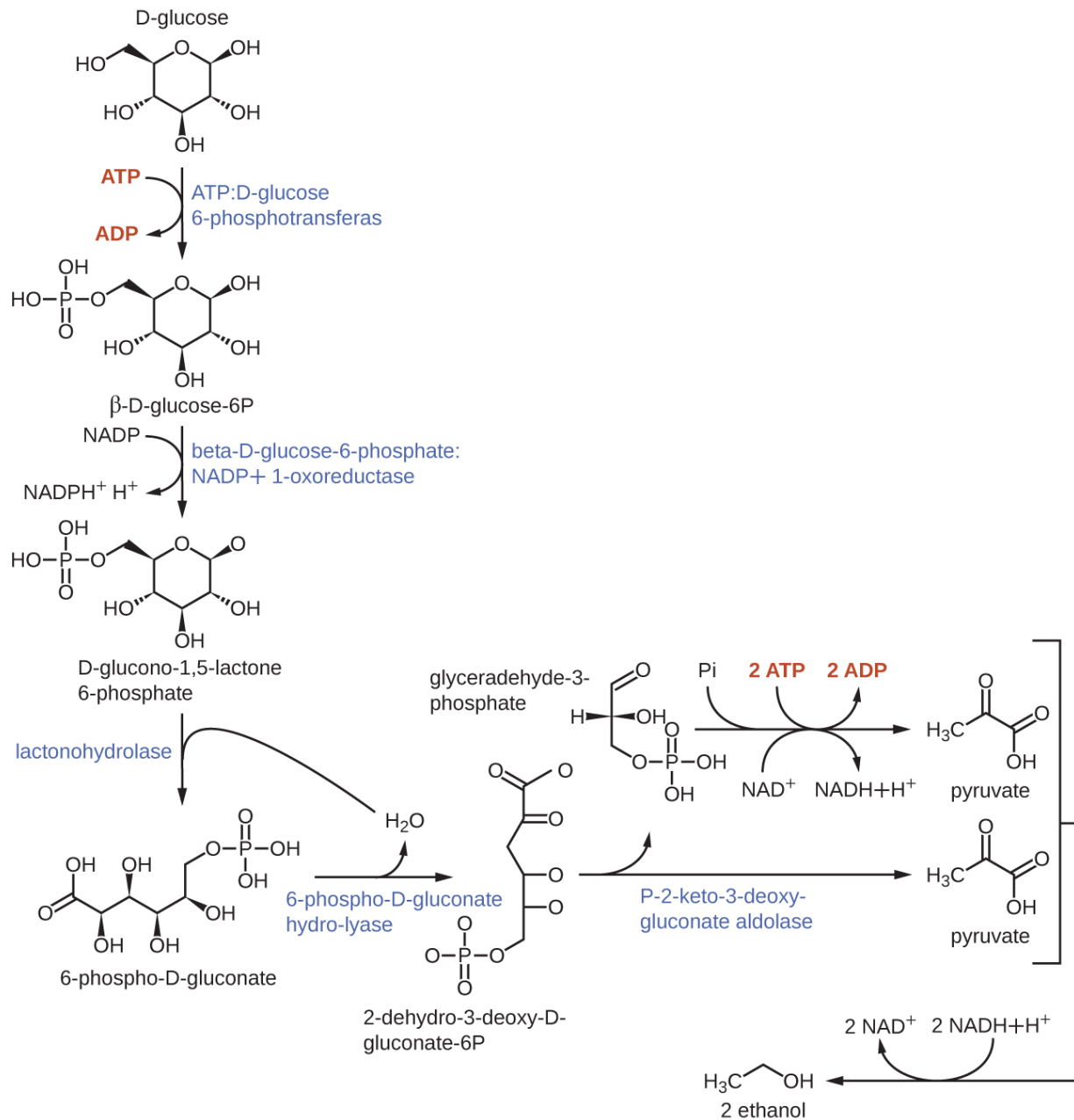


The first half of glycolysis uses two ATP molecules in the phosphorylation of glucose, which is then split into two three-carbon molecules.



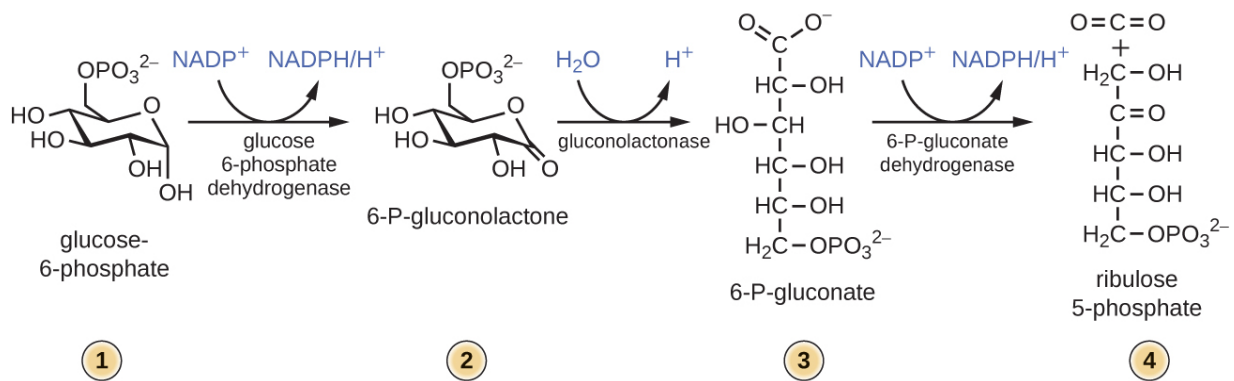
The second half of glycolysis involves phosphorylation without ATP investment (step 6) and produces two NADH and four ATP molecules per glucose.

Entner–Doudoroff Pathway



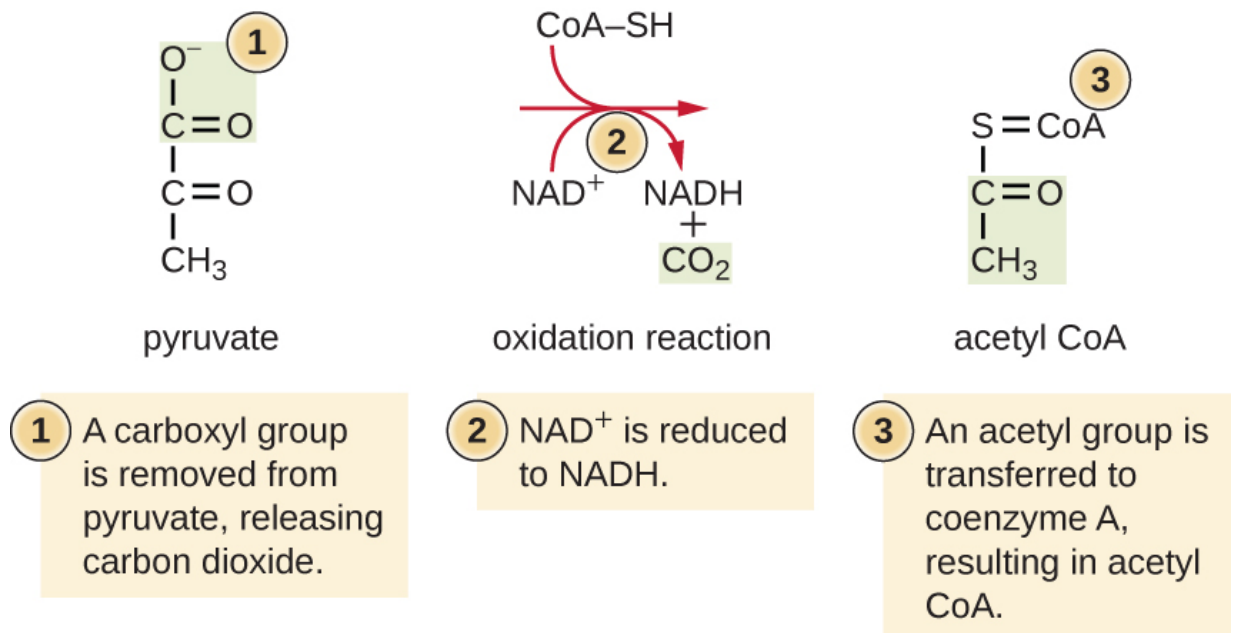
The Entner–Doudoroff Pathway is a metabolic pathway that converts glucose to ethanol and nets one ATP.

The Pentose-Phosphate Pathway



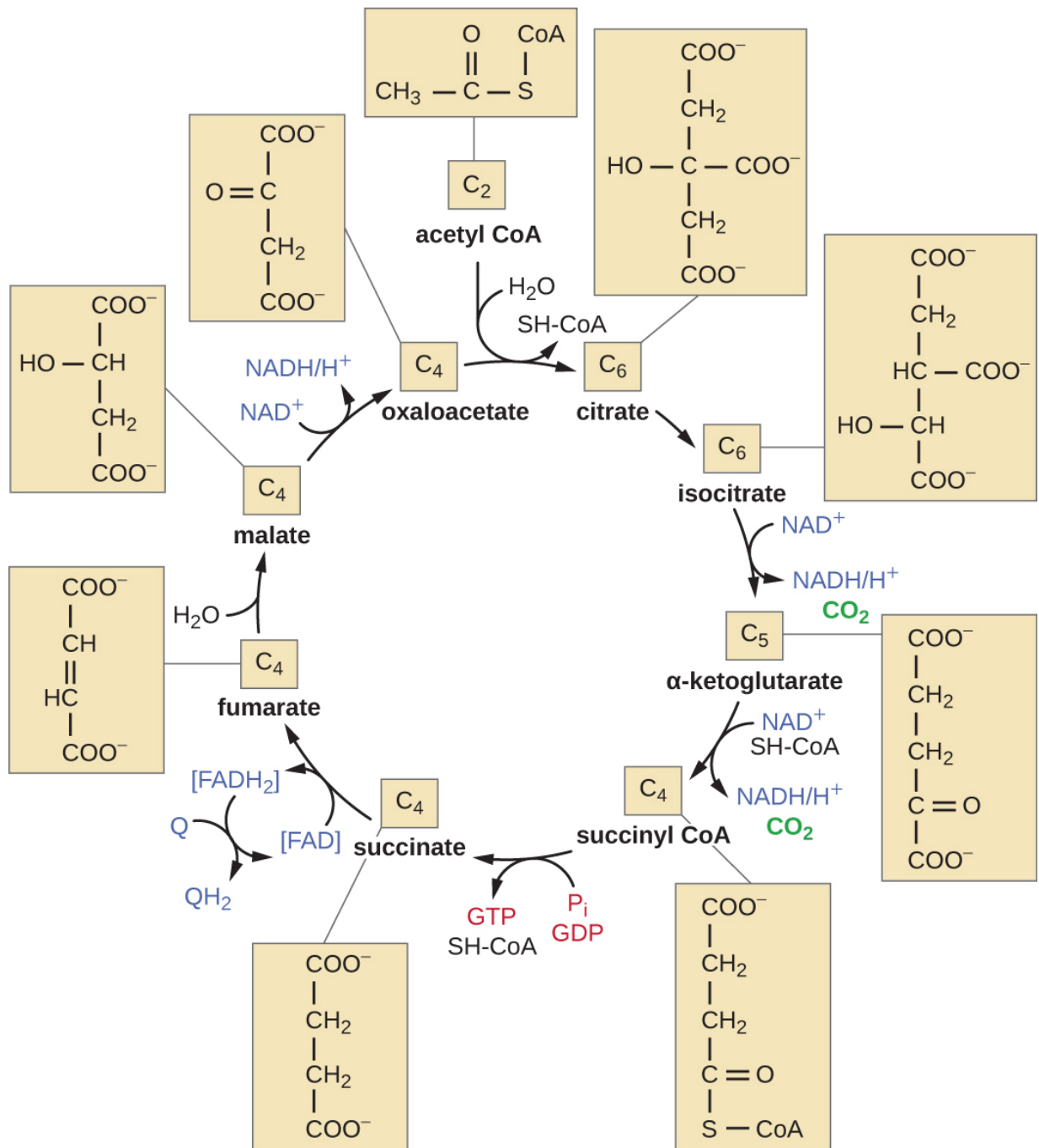
The pentose phosphate pathway, also called the phosphogluconate pathway and the hexose monophosphate shunt, is a metabolic pathway parallel to glycolysis that generates NADPH and five-carbon sugars as well as ribose 5-phosphate, a precursor for the synthesis of nucleotides from glucose.

TCA Cycle



In this transition reaction, a multi-enzyme complex converts pyruvate

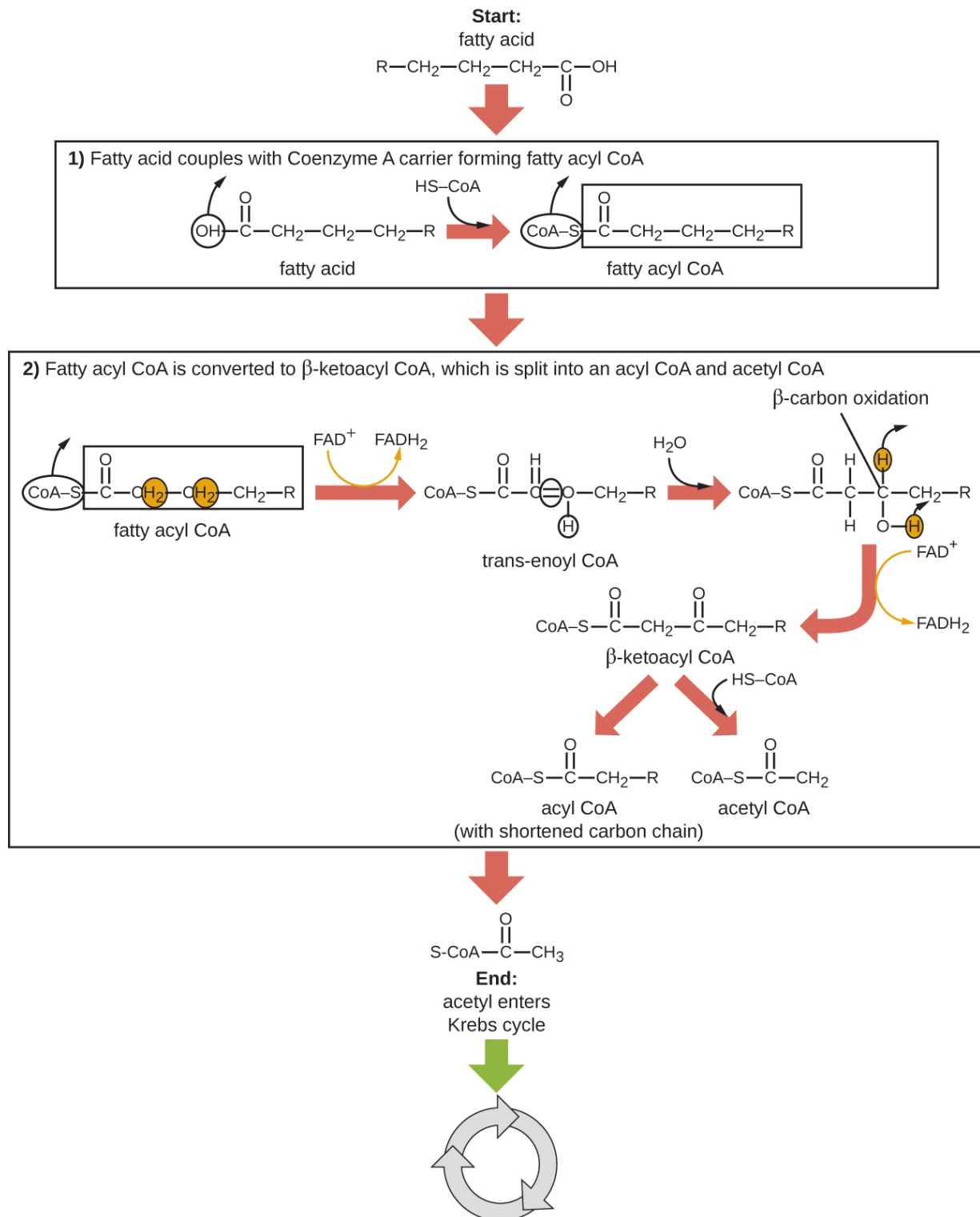
into one acetyl (2C) group plus one carbon dioxide (CO_2). The acetyl group is attached to a Coenzyme A carrier that transports the acetyl group to the site of the Krebs cycle. In the process, one molecule of NADH is formed.



In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NADH, one FADH₂, and one ATP or GTP (depending on the cell type) is produced by substrate-level phosphorylation. Because the final product of the citric acid cycle is

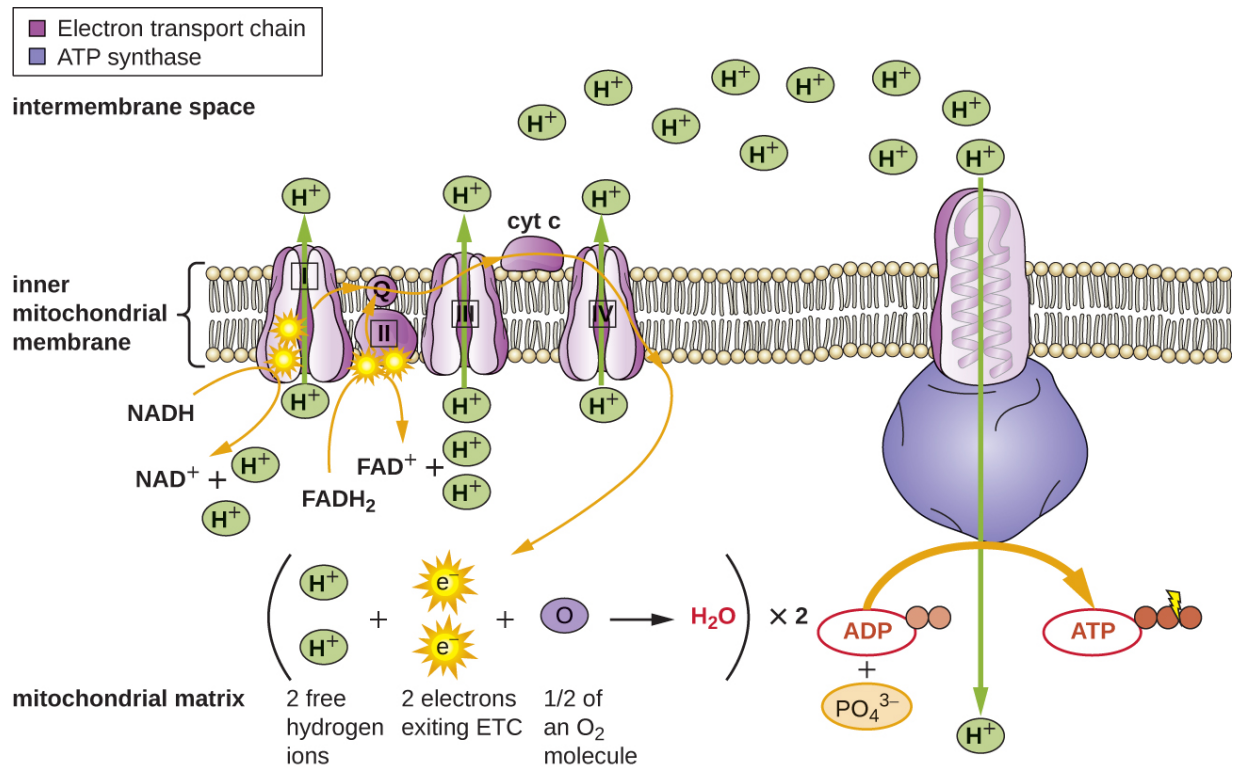
also the first reactant, the cycle runs continuously in the presence of sufficient reactants. (credit: modification of work by “Yikrazuul”/Wikimedia Commons)

Beta Oxidation



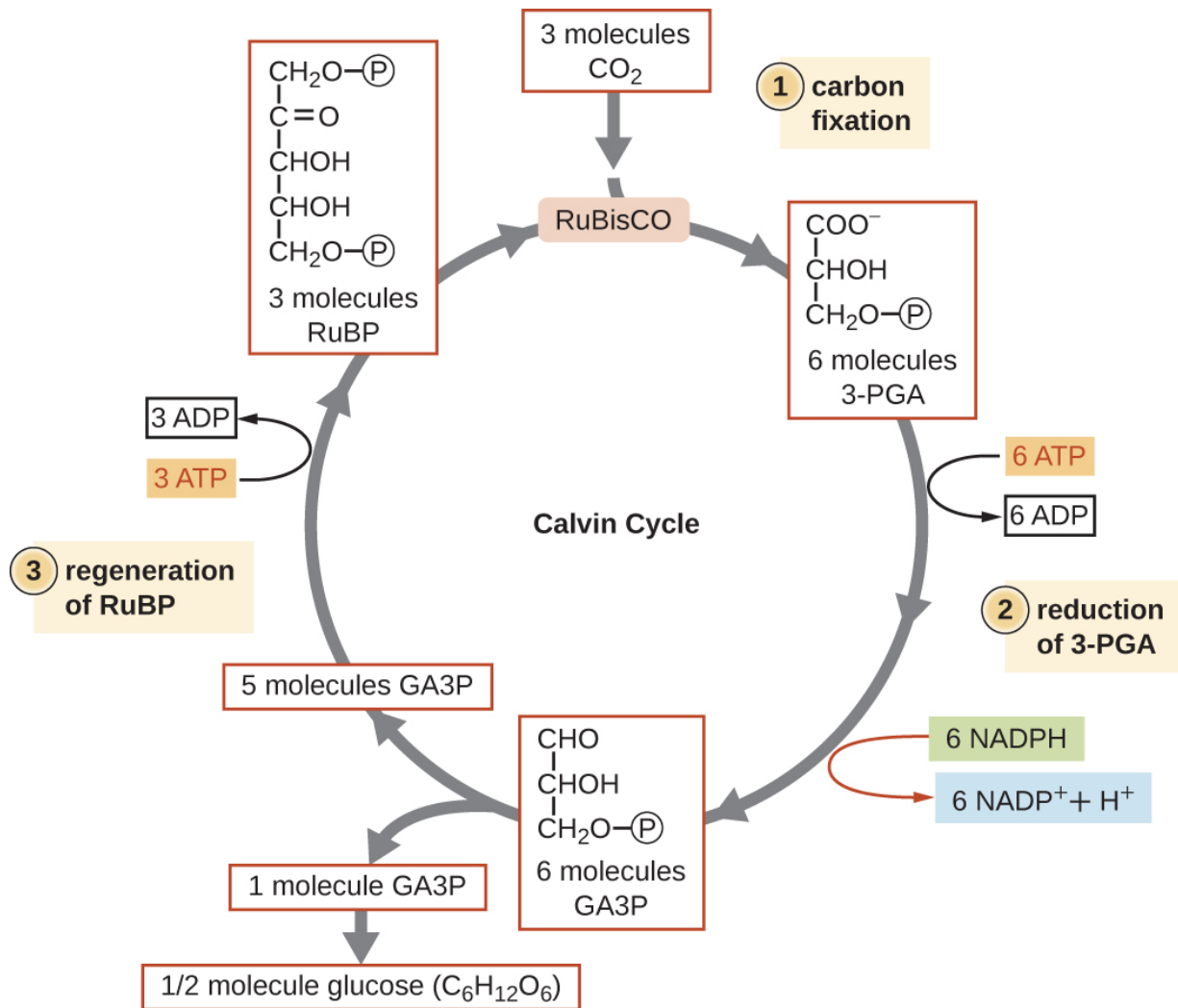
During fatty acid oxidation, triglycerides can be broken down into 2C acetyl groups that can enter the Krebs cycle and be used as a source of energy when glucose levels are low.

Electron Transport Chain and Oxidative Phosphorylation



The electron transport chain is a series of electron carriers and ion pumps that are used to pump H^+ ions across a membrane. H^+ then flow back through the membrane by way of ATP synthase, which catalyzes the formation of ATP. The location of the electron transport chain is the inner mitochondrial matrix in eukaryotic cells and cytoplasmic membrane in prokaryotic cells.

Calvin-Benson Cycle



The Calvin-Benson cycle has three stages. In stage 1, the enzyme RuBisCO incorporates carbon dioxide into an organic molecule, 3-PGA. In stage 2, the organic molecule is reduced using electrons supplied by NADPH. In stage 3, RuBP, the molecule that starts the cycle, is regenerated so that the cycle can continue. Only one carbon dioxide molecule is incorporated at a time, so the cycle must be completed three times to produce a single three-carbon GA3P molecule, and six times to produce a six-carbon glucose molecule.

Taxonomy of Clinically Relevant Microorganisms

Bacterial Pathogens

The following tables list the species, and some higher groups, of pathogenic Eubacteria mentioned in the text. The classification of Bacteria, one of the three domains of life, is in constant flux as relationships become clearer through sampling of genetic sequences. Many groups at all taxonomic levels still have an undetermined relationship with other members of the phylogenetic tree of Bacteria. *Bergey's Manual of Systematics of Archaea and Bacteria* maintains a published list and descriptions of prokaryotic species. The tables here follow the taxonomic organization in the *Bergey's Manual Taxonomic Outline*.[\[footnote\]](#)

Bergey's Manual Trust. *Bergey's Manual of Systematics of Archaea and Bacteria, Taxonomic Outline*. 2012. <http://www.bergeys.org/outlines.html>

We have divided the species into tables corresponding to different bacterial phyla. The taxonomic rank of kingdom is not used in prokaryote taxonomy, so the phyla are the subgrouping below domain. Note that many bacterial phyla not represented by these tables. The species and genera are listed only under the class within each phylum. The names given to bacteria are regulated by the International Code of Nomenclature of Bacteria as maintained by the International Committee on Systematics of Prokaryotes.

Phylum Actinobacteria			
Class	Genus	Species	Related Diseases
Actinobacteria	<i>Corynebacterium</i>	<i>diphtheriae</i>	Diphtheria
	<i>Gardnerella</i>	<i>vaginalis</i>	Bacterial vaginosis
	<i>Micrococcus</i>		Opportunistic infections
	<i>Mycobacterium</i>	<i>bovis</i>	Tuberculosis, primarily in cattle
	<i>Mycobacterium</i>	<i>leprae</i>	Hansen's disease
	<i>Mycobacterium</i>	<i>tuberculosis</i>	Tuberculosis
	<i>Propionibacterium</i>	<i>acnes</i>	Acne, blepharitis, endophthalmitis

Phylum Bacteroidetes			
Class	Genus	Species	Related Diseases
Bacteroidia	<i>Porphyromonas</i>		Periodontal disease
	<i>Prevotella</i>	<i>intermedia</i>	Periodontal disease

Phylum Chlamydiae			
Class	Genus	Species	Related Diseases
Chlamydiae	<i>Chlamydia</i>	<i>psittaci</i>	Psittacosis
	<i>Chlamydia</i>	<i>trachomatis</i>	Sexually transmitted chlamydia

Phylum Firmicutes			
Class	Genus	Species	Related Diseases
Bacilli	<i>Bacillus</i>	<i>anthracis</i>	Anthrax
	<i>Bacillus</i>	<i>cereus</i>	Diarrheal and emetic food poisoning
	<i>Listeria</i>	<i>monocytogenes</i>	Listeriosis
	<i>Enterococcus</i>	<i>faecalis</i>	Endocarditis, septicemia, urinary tract infections, meningitis
	<i>Staphylococcus</i>	<i>aureus</i>	Skin infections, sinusitis, food poisoning

Phylum Firmicutes			
Class	Genus	Species	Related Diseases
	<i>Staphylococcus</i>	<i>epidermidis</i>	Nosocomial and opportunistic infections
	<i>Staphylococcus</i>	<i>hominis</i>	Opportunistic infections
	<i>Staphylococcus</i>	<i>saprophyticus</i>	Urinary tract infections
	<i>Streptococcus</i>	<i>agalactiae</i>	Postpartum infection, neonatal sepsis
	<i>Streptococcus</i>	<i>mutans</i>	Tooth decay
	<i>Streptococcus</i>	<i>pneumoniae</i>	Pneumonia, many other infections
	<i>Streptococcus</i>	<i>pyogenes</i>	Pharyngitis, scarlet fever, impetigo, necrotizing fasciitis
Clostridia	<i>Clostridium</i>	<i>botulinum</i>	Botulinum poisoning
	<i>Clostridium</i>	<i>difficile</i>	Colitis
	<i>Clostridium</i>	<i>perfringens</i>	Food poisoning, gas gangrene
	<i>Clostridium</i>	<i>tetani</i>	Tetanus

Phylum Fusobacteria			
Class	Genus	Species	Related Diseases
Fusobacteriia	<i>Fusobacterium</i>		Periodontal disease, Lemierre syndrome, skin ulcers
	<i>Streptobacillus</i>	<i>moniliformis</i>	Rat-bite fever

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
Alphaproteobacteria	<i>Anaplasma</i>	<i>phagocytophilum</i>	Human granulocytic anaplasmosis
	<i>Bartonella</i>	<i>henselae</i>	Peliosis hepatitis, bacillary angiomatosis, endocarditis, bacteremia
	<i>Bartonella</i>	<i>quintana</i>	Trench fever
	<i>Brucella</i>	<i>melitensis</i>	Ovine brucellosis
	<i>Ehrlichia</i>	<i>chaffeensis</i>	Human monocytic ehrlichiosis
	<i>Rickettsia</i>	<i>prowazekii</i>	Epidemic typhus
	<i>Rickettsia</i>	<i>rickettsii</i>	Rocky Mountain spotted fever
	<i>Rickettsia</i>	<i>typhi</i>	Murine typhus
Betaproteobacteria	<i>Bordetella</i>	<i>pertussis</i>	Pertussis
	<i>Eikenella</i>		Bite-injury infections
	<i>Neisseria</i>	<i>gonorrhoeae</i>	Gonorrhea
	<i>Neisseria</i>	<i>meningitidis</i>	Meningitis

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Spirillum</i>	<i>minus (alt. minor)</i>	Sodoku (rat-bite fever)
Epsilonproteobacteria	<i>Campylobacter</i>	<i>jejuni</i>	Gastroenteritis, Guillain-Barré syndrome
	<i>Helicobacter</i>	<i>pylori</i>	Gastric ulcers
Gammaproteobacteria	<i>Aeromonas</i>	<i>hydrophila</i>	Dysenteric gastroenteritis
	<i>Coxiella</i>	<i>burnetii</i>	Q fever
	<i>Enterobacter</i>		Urinary and respiratory infections
	<i>Escherichia</i>	<i>coli</i> Strains: shiga toxin-producing (STEC) (e.g., O157:H7) also called enterohemorrhagic <i>E. coli</i> (EHEC) or verocytotoxin-producing <i>E. coli</i> (VTEC)	Foodborne diarrhea outbreaks, hemorrhagic colitis, hemolytic-uremic syndrome
	<i>Escherichia</i>	<i>coli</i> Strain: enterotoxigenic <i>E. coli</i> (ETEC)	Traveler's diarrhea
	<i>Escherichia</i>	<i>coli</i> Strain: enteropathogenic <i>E. coli</i> (EPEC)	Diarrhea, especially in young children

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Escherichia</i>	<i>coli</i> Strain: enteroaggregative <i>E. coli</i> (EAEC)	Diarrheal disease in children and travelers
	<i>Escherichia</i>	<i>coli</i> Strain: diffusely adherent <i>E. coli</i> (DAEC)	Diarrheal disease of children
	<i>Escherichia</i>	<i>coli</i> Strain: enteroinvasive <i>E. coli</i> (EPEC)	Bacillary dysentery, cells invade intestinal epithelial cells
	<i>Francisella</i>	<i>tularensis</i>	Tularemia
	<i>Haemophilus</i>	<i>ducreyi</i>	Chancroid
	<i>Haemophilus</i>	<i>influenzae</i>	Bacteremia, pneumonia, meningitis
	<i>Klebsiella</i>	<i>pneumoniae</i>	Pneumonia, nosocomial infections
	<i>Legionella</i>	<i>pneumophila</i>	Legionnaire's disease
	<i>Moraxella</i>	<i>catarrhalis</i>	Otitis media, bronchitis, sinusitis, laryngitis, pneumonia
	<i>Pasteurella</i>		Pasteurellosis

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Plesiomonas</i>	<i>shigelloides</i>	Gastroenteritis
	<i>Proteus</i>		Opportunistic urinary tract infections
	<i>Pseudomonas</i>	<i>aeruginosa</i>	Opportunistic, nosocomial pneumonia and sepsis
	<i>Salmonella</i>	<i>bongori</i>	Salmonellosis
	<i>Salmonella</i>	<i>enterica</i>	Salmonellosis
	<i>Serratia</i>		Pneumonia, urinary tract infections
	<i>Shigella</i>	<i>boydii</i>	Dysentery
	<i>Shigella</i>	<i>dysenteriae</i>	Dysentery
	<i>Shigella</i>	<i>flexneri</i>	Dysentery
	<i>Shigella</i>	<i>sonnei</i>	Dysentery
	<i>Vibrio</i>	<i>cholerae</i>	Cholera
	<i>Vibrio</i>	<i>parahemolyticus</i>	Seafood gastroenteritis
	<i>Vibrio</i>	<i>vulnificus</i>	Seafood gastroenteritis, necrotizing wound infections, septicemia

Phylum Proteobacteria			
Class	Genus	Species	Related Diseases
	<i>Yersinia</i>	<i>enterocolitica</i>	Yersiniosis
	<i>Yersinia</i>	<i>pestis</i>	Plague
	<i>Yersinia</i>	<i>pseudotuberculosis</i>	Far East scarlet-like fever

Phylum Spirochaetes			
Class	Genus	Species	Related Diseases
Spirochaetia	<i>Borrelia</i>	<i>burgdorferi</i>	Lyme disease
	<i>Borrelia</i>	<i>hermsii</i>	Tick-borne relapsing fever
	<i>Borrelia</i>	<i>recurrentis</i>	Louse-borne relapsing fever
	<i>Leptospira</i>	<i>interrogans</i>	Leptospirosis
	<i>Treponema</i>	<i>pallidum</i>	Syphilis, bejel, pinta, yaws

Phylum Tenericutes			
Class	Genus	Species	Related Diseases
Mollicutes	<i>Mycoplasma</i>	<i>genitalium</i>	Urethritis, cervicitis

Phylum Tenericutes			
Class	Genus	Species	Related Diseases
	<i>Mycoplasma</i>	<i>hominis</i>	Pelvic inflammatory disease, bacterial vaginosis
	<i>Mycoplasma</i>	<i>pneumoniae</i>	<i>Mycoplasma pneumonia</i>
	<i>Ureaplasma</i>	<i>urealyticum</i>	Urethritis, fetal infections

Viral Pathogens

There are several classification systems for viruses. The International Committee on Taxonomy of Viruses (ICTV) is the international scientific body responsible for the rules of viral classification. The ICTV system used here groups viruses based on genetic similarity and presumed monophyly. The viral classification system is separate from the classification system for cellular organisms. The ICTV system groups viruses within seven orders, which contain related families. There is, presently, a large number of unassigned families with unknown affinities to the seven orders. Three of these orders infect only Eubacteria, Archaea, or plants and do not appear in this table. Some families may be divided into subfamilies. There are also many unassigned genera. Like all taxonomies, viral taxonomy is in constant flux. The latest complete species list and classification can be obtained on the ICTV website.[\[footnote\]](#)

International Committee on Taxonomy of Viruses. "ICTV Master Species List."

http://talk.ictvonline.org/files/ictv_documents/m/msl/default.aspx

Viral Pathogens					
Order	Family	Sub-family	Genus	Species	Related diseases
<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Betaherpesvirinae</i>	<i>Human cytomegalovirus group</i>	<i>Human herpesvirus 5</i>	Cytomegalovirus hepatitis and other infections in immunocompromised people
		<i>Gammapherpesvirinae</i>	<i>Lymphocryptovirus</i>	<i>Human herpesvirus 4 (HHV-4; Epstein-Barr virus)</i>	Infectious mononucleosis
		<i>Alphaherpesvirinae</i>	<i>Simplexvirus</i>	<i>Human herpesvirus 1, human herpesvirus 2</i>	Herpes simplex virus 1, herpes simplex virus 2
			<i>Varicellovirus</i>	<i>Human herpesvirus 3</i>	Chicken pox, shingles
<i>Mononegavirales</i>	<i>Filoviridae</i>		<i>Ebolavirus</i>	<i>Zaire ebolavirus (EBOV)</i>	Ebola
			<i>Marburgvirus</i>	<i>Marburg marburgvirus (MARV)</i>	Marburg virus disease
	<i>Rhabdoviridae</i>		<i>Lyssavirus</i>	<i>Rabies virus</i>	Rabies
	<i>Paramyxoviridae</i>	<i>Pneumovirinae</i>	<i>Pneumovirus</i>	<i>Human respiratory syncytial virus</i>	Lower respiratory tract infection
		<i>Paramyxovirinae</i>	<i>Morbillivirus</i>	<i>Measles virus</i>	Measles (rubeola)
<i>Nidovirales</i>	<i>Coronaviridae</i>	<i>Coronavirinae</i>	<i>Coronavirus</i>		Common cold, pneumonia, SARS
<i>Picornavirales</i>	<i>Picornaviridae</i>		<i>Hepatovirus</i>	<i>Hepatitis A virus</i>	Hepatitis A
			<i>Enterovirus</i>	<i>Enterovirus C</i>	Polio
				<i>Rhinovirus A</i>	Common cold
				<i>Rhinovirus B</i>	Common cold
				<i>Rhinovirus C</i>	Common cold
Unassigned	<i>Adenovirus</i>		<i>Mastadenovirus</i>		Respiratory and other infections
	<i>Arenaviridae</i>		<i>Mammarenavirus</i>	<i>Lassa mammarenavirus</i>	Lassa fever
	<i>Astroviridae</i>				Gastroenteritis
	<i>Bunyaviridae</i>		<i>Hantavirus</i>	Several species	Hantavirus hemorrhagic fever with renal syndrome (HFRS), hantavirus pulmonary syndrome (HPS)
			<i>Nairovirus</i>	<i>Crimean-Congo hemorrhagic fever virus (CCHF)</i>	Crimean-Congo hemorrhagic fever
	<i>Caliciviridae</i>		<i>Norovirus</i>	<i>Norwalk virus</i>	Gastroenteritis

Viral Pathogens (continued)					
Order	Family	Sub-family	Genus	Species	Related diseases
Unassigned	<i>Flaviviridae</i>		<i>Flavivirus</i>	<i>Dengue virus</i>	Dengue fever
				<i>Yellow fever virus</i>	Yellow fever
			<i>Hepacivirus</i>	<i>Hepatitis C virus</i>	Hepatitis C
	<i>Hepadnaviridae</i>		<i>Orthohepadnavirus</i>	<i>Hepatitis B virus</i>	Hepatitis B
	<i>Hepeviridae</i>		<i>Orthohepevirus</i>	<i>Hepatitis E virus</i>	Hepatitis E
	<i>Orthomyxoviridae</i>		<i>Influenzavirus A</i>	<i>Influenza A virus</i>	Pandemic flu
			<i>Influenzavirus B</i>	<i>Influenza B virus</i>	Flu
			<i>Influenzavirus C</i>	<i>Influenza C virus</i>	Flu
	<i>Papillomaviridae</i>		<i>Alphapapillomavirus</i>	<i>Human papillomavirus</i>	Skin warts
	<i>Parvoviridae</i>	<i>Parvovirinae</i>	<i>Erythroparvovirus</i>	<i>Human parvovirus B19</i>	Fifth disease (erythema infectiosum)
	<i>Poxviridae</i>	<i>Chordopoxvirinae</i>	<i>Orthopoxvirus</i>	<i>Variola virus</i>	Variola major, Variola minor (smallpox)
				<i>Vaccinia virus</i>	Cowpox
	<i>Reoviridae</i>	<i>Sedoreovirinae</i>	<i>Rotavirus</i>	Eight species	Gastroenteritis
	<i>Retroviridae</i>	<i>Orthoretrovirinae</i>	<i>Lentivirus</i>	<i>Human immunodeficiency virus</i>	AIDS
	<i>Togaviridae</i>		<i>Alphavirus</i>	<i>Chikungunya virus (CHIKV)</i>	Chikungunya
			<i>Rubivirus</i>	<i>Rubella virus</i>	Rubella (German measles)
	Unassigned		<i>Deltavirus</i>	<i>Hepatitis D virus</i>	Hepatitis D

Fungal Pathogens

The Fungi are one of the kingdoms of the domain Eukarya. Fungi are most closely related to the animals and a few other small groups and more distantly related to the plants and other groups that formerly were categorized as protist. At present, the Fungi are divided into seven phyla (or divisions, a hold over from when fungi were studied with plants), but there are uncertainties about some relationships.^[footnote] Many groups of fungi, particularly those that were formerly classified in the phylum Zygomycota, which was not monophyletic, have uncertain relationships to the other fungi. The one species listed in this table that falls into this category is *Rhizopus arrhizus*. Fungal names are governed by the International Code of Nomenclature for Algae, Fungi, and Plants,^[footnote] but the International Commission on the Taxonomy of Fungi (ICTF) also promotes taxonomic work on fungi. One activity of the ICTF is publicizing name changes for medically and otherwise important fungal species. Many species that formerly had two names (one for the sexual form and one for the asexual form) are now being brought together under one name.

D. S. Hibbett et al. "A Higher-level Phylogenetic Classification of the Fungi." *Mycological Research* 111 no. 5 (2007):509–547.

J. McNeill et al. *International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code)*. Oberreifener, Germany. Koeltz Scientific Books; 2012. <http://www.iapt-taxon.org/nomen/main.php>

Fungal Pathogens			
Division	Genus	Species	Related Diseases
Ascomycota	<i>Aspergillus</i>	<i>flavus</i>	Opportunistic aspergillosis
	<i>Aspergillus</i>	<i>fumigatus</i>	Opportunistic aspergillosis
	<i>Blastomyces</i>	<i>dermatitidis</i>	Blastomycosis
	<i>Candida</i>	<i>albicans</i>	Thrush (candidiasis)
	<i>Coccidioides</i>	<i>immitis</i>	Valley fever (coccidioidomycosis)
	<i>Epidermophyton</i>		Tinea corporis (ringworm), tinea cruris (jock itch), tinea pedis (athlete's foot), tinea unguium (onychomycosis)
	<i>Histoplasma</i>	<i>capsulatum</i>	Histoplasmosis
	<i>Microsporum</i>		Tinea capitis (ringworm), tinea corpus (ringworm), other dermatophytoses
	<i>Pneumocystis</i>	<i>jirovecii</i>	Opportunistic pneumonia
	<i>Sporothrix</i>	<i>schenckii</i>	Sporotrichosis (rose-handler's disease)

Fungal Pathogens			
Division	Genus	Species	Related Diseases
	<i>Trichophyton</i>	<i>mentagrophytes</i> var. <i>interdigitale</i>	Tinea barbae (barber's itch), dermatophytoses
	<i>Trichophyton</i>	<i>rubrum</i>	Tinea corporis (ringworm), tinea cruris (jock itch), tinea pedis (athlete's foot), tinea unguium (onychomycosis)
Basidiomycota	<i>Cryptococcus</i>	<i>neoformans</i>	Opportunistic cryptococcosis, fungal meningitis, encephalitis
	<i>Malassezia</i>		Dandruff, tinea versicolor
uncertain	<i>Rhizopus</i>	<i>arrhizus</i>	Mucormycosis

Protozoan Pathogens

The relationships among the organisms (and thus their taxonomy) previously grouped under the name Protists are better understood than they were two or three decades ago, but this is still a work in progress. In 2005, the Eukarya were divided into six supergroups.^[footnote] The latest high-level classification combined two of the previous supergroups to produce a system comprising five supergroups.^[footnote] This classification was developed for the Society of Protozoologists, but it is not the only suggested approach. One of the five supergroups includes the animals, fungi, and some smaller protist groups. Another contains green plants and three algal groups. The other three supergroups (listed in the three tables below) contain the other protists, many of them which cause disease. In addition, there is a large number of protist groups whose relationships are not understood. In the three supergroups represented here we have indicated the phyla to which the listed pathogens belong.

S.M. Adl et al. "The New Higher Level Classification of Eukaryotes with Emphasis on the Taxonomy of Protists." *Journal of Eukaryotic Microbiology* 52 no. 5 (2005):399–451.

S.M. Adl et al. "The Revised Classification of Eukaryotes." *Journal of Eukaryotic Microbiology* 59 no. 5 (2012):429–514.

Supergroup Amoebozoa			
Phylum	Genus	Species	Related Diseases
Amoebozoa	<i>Acanthamoeba</i>		Granulomatous amoebic encephalitis, acanthamoebic keratitis
	<i>Entamoeba</i>	<i>histolytica</i>	Enterobiasis

Supergroup SAR (Stramenopiles, Alveolata, Rhizaria)			
Phylum	Genus	Species	Related Diseases
Apicomplexa	<i>Babesia</i>		Babesiosis
	<i>Cryptosporidium</i>	<i>hominis</i>	Cryptosporidiosis
	<i>Cryptosporidium</i>	<i>parvum</i>	Cryptosporidiosis
	<i>Cyclospora</i>	<i>cayetanensis</i>	Gastroenteritis
	<i>Plasmodium</i>	<i>falciparum</i>	Malaria
	<i>Plasmodium</i>	<i>malariae</i>	“Benign” or “quartan” (3-day recurrent fever) malaria
	<i>Plasmodium</i>	<i>ovale</i>	“Tertian” (2-day recurrent fever) malaria
	<i>Plasmodium</i>	<i>vivax</i>	“Benign” “tertian” (2-day recurrent fever) malaria
	<i>Plasmodium</i>	<i>knowlesi</i>	Primate malaria capable of zoonosis, quotidian fever
	<i>Toxoplasma</i>	<i>gondii</i>	Toxoplasmosis

Supergroup Excavata			
Phylum	Genus	Species	Related Diseases
Metamonada	<i>Giardia</i>	<i>lamblia</i>	Giardiasis
	<i>Trichomonas</i>	<i>vaginalis</i>	Trichomoniasis
Euglenozoa	<i>Leishmania</i>	<i>braziliensis</i>	Leishmaniasis
	<i>Leishmania</i>	<i>donovani</i>	Leishmaniasis
	<i>Leishmania</i>	<i>tropica</i>	Cutaneous leishmaniasis
	<i>Trypanosoma</i>	<i>brucei</i>	African sleeping sickness (African trypanosomiasis)
	<i>Trypanosoma</i>	<i>cruzi</i>	Chagas disease
Percolozoa	<i>Naegleria</i>	<i>fowleri</i>	Primary amoebic meningoencephalitis (naegleriasis)

Parasitic Helminths

The taxonomy of parasitic worms, all of which belong to the kingdom Animalia still contains many uncertainties. The pathogenic species are found in two phyla: the Nematoda, or roundworms, and the Platyhelminthes, or flat worms. The Nematoda is tentatively divided into two classes^[footnote], one of which, Chromadorea, probably contains unrelated groups. The parasitic flatworms are contained within three classes of flatworm, of which two are important to humans, the trematodes and the cestodes.

National Center for Biotechnology Information. "Taxonomy Browser: Nematoda."
<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=6231>

Phylum Nematoda			
Class	Genus	Species	Related Diseases
Chromadorea	<i>Ancylostoma</i>	<i>caninum</i>	Dog hookworm infection

Phylum Nematoda			
Class	Genus	Species	Related Diseases
	<i>Ancylostoma</i>	<i>duodenale</i>	Old World hookworm infection
	<i>Ascaris</i>	<i>lumbricoides</i>	Ascariasis
	<i>Enterobius</i>	<i>vermicularis</i>	Enterobiasis (pin worm)
	<i>Loa</i>	<i>loa</i>	Loa loa filariasis (eye worm)
	<i>Necator</i>	<i>americanus</i>	Necatoriasis (New World hookworm infection)
	<i>Strongyloides</i>	<i>stercoralis</i>	Strongyloidiasis
Enoplea	<i>Trichinella</i>	<i>spiralis</i>	Trichinosis
	<i>Trichuris</i>	<i>trichiura</i>	Trichuriasis (whip worm infection)

Phylum Platyhelminthes			
Class	Genus	Species	Related Diseases
Trematoda	<i>Clonorchis</i>	<i>sinensis</i>	Chinese liver fluke
	<i>Fasciolopsis</i>	<i>buski</i>	Fasciolopsiasis
	<i>Fasciola</i>	<i>gigantica</i>	Fascioliasis
	<i>Fasciola</i>	<i>hepatica</i>	Fascioliasis
	<i>Opisthorchis</i>	<i>felineus</i>	Opisthorchiasis
	<i>Opisthorchis</i>	<i>viverrini</i>	Opisthorchiasis

Phylum Platyhelminthes			
Class	Genus	Species	Related Diseases
	<i>Schistosoma</i>	<i>haematobium</i>	Urinary schistosomiasis
	<i>Schistosoma</i>	<i>japonicum</i>	Schistosomiasis
	<i>Schistosoma</i>	<i>mansoni</i>	Intestinal schistosomiasis
Cestoda	<i>Diphyllobothrium</i>	<i>latum</i>	Diphyllobothriosis
	<i>Echinococcus</i>	<i>granulosus</i>	Hydatid cysts (cystic echinococcosis)
	<i>Echinococcus</i>	<i>multilocularis</i>	Echinococcosis
	<i>Taenia</i>	<i>asiatica</i>	Intestinal taeniasis
	<i>Taenia</i>	<i>saginata</i>	Intestinal taeniasis
	<i>Taenia</i>	<i>solium</i>	Intestinal taeniasis, cysticercosis

Glossary

- **454 sequencing (pyrosequencing)** a next generation sequencing technique in which fragmented DNA has DNA adapters attached, is amplified by PCR, is attached to a bead, and then placed into a well with sequencing reagents, and the flash of light produced by the release of pyrophosphate on addition of a nucleotide is monitored
- **5' cap** methylguanosine nucleotide added to 5' end of a eukaryotic primary transcript
- **70S ribosome** a ribosome composed of 50S and 30S subunits
- **80S ribosome** cytoplasmic eukaryotic ribosome composed of 60S and 40S subunits

A

- **α -helix** secondary structure consisting of a helix stabilized by hydrogen bonds between nearby amino acid residues in a polypeptide
- **A (aminoacyl) site** functional site of an intact ribosome that binds incoming charged aminoacyl tRNAs
- **A-B exotoxin** class of exotoxin that contains A subunits, which enter the cell and disrupt cellular activities, and B subunits, which bind to host cell receptors
- **ABO blood group system** set of glycoprotein antigens found on the surface of red blood cells; the presence or absence of specific carbohydrates determining blood type
- **absorbance** when a molecule captures energy from a photon and vibrates or stretches, using the energy
- ***Acanthamoeba keratitis*** a condition characterized by damage to the cornea and possible blindness caused by parasitic infection of the protozoan *Acanthamoeba*
- **acellular** not made of cells
- **acid-fast stain** a stain that differentiates cells that have waxy mycolic acids in their gram-positive cell walls

- **acidic dye** a chromophore with a negative charge that attaches to positively charged structures
- **acidophile** organism that grows optimally at a pH near 3.0
- **acne** a skin disease in which hair follicles or pores become clogged, leading to the formation of comedones and infected lesions
- **acquired immunodeficiency syndrome (AIDS)** disease caused by HIV, characterized by opportunistic infections and rare cancers
- **actin** a protein that polymerizes to form microfilaments
- **activation energy** energy needed to form or break chemical bonds and convert a reactant or reactants to a product or products
- **activator** protein that increases the transcription of a gene in response to an external stimulus
- **active carrier** an infected individual who can transmit the pathogen to others regardless of whether symptoms are currently present
- **active immunity** stimulation of one's own adaptive immune responses
- **active site** location within an enzyme where substrate(s) bind
- **acute disease** disease of a relatively short duration that develops and progresses in a predictable pattern
- **acute glomerulonephritis** inflammation of the glomeruli of the kidney, probably resulting from deposition of immune complexes and an autoimmune response caused by self-antigen mimicry by a pathogen
- **acute necrotizing ulcerative gingivitis** a severe form of gingivitis, also called trench mouth
- **acute otitis media** inflammatory disease of the middle ear resulting from a microbial infection
- **acute rheumatic fever** sequela of streptococcal pharyngitis; comorbidities include arthritis and carditis
- **acute-phase proteins** antimicrobial molecules produced by liver cells in response to pathogen-induced stimulation events
- **acyclovir** antiviral guanosine analog; inhibits DNA replication
- **adaptive immunity** third-line defense characterized by specificity and memory
- **Addison disease** autoimmune disease affecting adrenal gland function
- **adenine** purine nitrogenous base found in nucleotides
- **adenosine diphosphate (ADP)** nucleotide derivative and relative of ATP containing only one high-energy phosphate bond

- **adenosine monophosphate (AMP)** adenine molecule bonded to a ribose molecule and to a single phosphate group, having no high-energy phosphate bonds
- **adenosine triphosphate (ATP)** energy currency of the cell; a nucleotide derivative that safely stores chemical energy in its two high-energy phosphate bonds
- **adhesins** molecules on the surface of pathogens that promote colonization of host tissue
- **adhesion** the capability of microbes to attach to host cells
- **aerobic respiration** use of an oxygen molecule as the final electron acceptor of the electron transport system
- **aerotolerant anaerobe** organism that does not use oxygen but tolerates its presence
- **affinity maturation** function of the immune system by which B cells, upon re-exposure to antigen, are selected to produce higher affinity antibodies
- **affinity** measure of how tightly an antibody-binding site binds to its epitope
- **aflatoxin** chemical produced by the fungus *Aspergillus flavus*; both a toxin and the most potent known natural carcinogen
- **African sleeping sickness** see *human African trypanosomiasis*
- **agarose gel electrophoresis** a method for separating populations of DNA molecules of varying sizes by differential migration rates caused by a voltage gradient through a horizontal gel matrix
- **agglutination** binding of different pathogen cells by Fab regions of the same antibody to aggregate and enhance elimination from body
- **agranulocytes** leukocytes that lack granules in the cytoplasm
- **alarmone** small intracellular derivative of a nucleotide that signals a global bacterial response (i.e., activating a regulon of operons) to an environmental stress
- **albendazole** antihelminthic drug of the benzimidazole class that binds to helminthic β -tubulin, preventing microtubule formation
- **algae** (singular: alga) any of various unicellular and multicellular photosynthetic eukaryotic organisms; distinguished from plants by their lack of vascular tissues and organs
- **alkaliphile** organism that grows optimally at pH above 9.0

- **alkylating agent** type of strong disinfecting chemical that acts by replacing a hydrogen atom within a molecule with an alkyl group, thereby inactivating enzymes and nucleic acids
- **allergen** antigen capable of inducing type I hypersensitivity reaction
- **allergy** hypersensitivity response to an allergen
- **allograft** transplanted tissue from an individual of the same species that is genetically different from the recipient
- **allosteric activator** molecule that binds to an enzyme's allosteric site, increasing the affinity of the enzyme's active site for the substrate(s)
- **allosteric site** location within an enzyme, other than the active site, to which molecules can bind, regulating enzyme activity
- **allylamines** class of antifungal drugs that inhibit ergosterol biosynthesis at an early point in the pathway
- **Alphaproteobacteria** class of Proteobacteria that are all oligotrophs
- **alveoli** cul-de-sacs or small air pockets within the lung that facilitate gas exchange
- **amantadine** antiviral drug that targets the influenza virus by preventing viral escape from endosomes upon host cell uptake, thus preventing viral RNA release and subsequent viral replication
- **amensalism** type of symbiosis in which one population harms the other but remains unaffected itself
- **Ames test** method that uses auxotrophic bacteria to detect mutations resulting from exposure to potentially mutagenic chemical compounds
- **amino acid** a molecule consisting of a hydrogen atom, a carboxyl group, and an amine group bonded to the same carbon. The group bonded to the carbon varies and is represented by an *R* in the structural formula
- **aminoacyl-tRNA synthetase** enzyme that binds to a tRNA molecule and catalyzes the addition of the correct amino acid to the tRNA
- **aminoglycosides** protein synthesis inhibitors that bind to the 30S subunit and interfere with the ribosome's proofreading ability, leading to the generation of faulty proteins that insert into and disrupt the bacterial cytoplasmic membrane
- **amoebiasis** intestinal infection caused by *Entamoeba histolytica*
- **amoebic dysentery** severe form of intestinal infection caused by *Entamoeba histolytica*, characterized by severe diarrhea with blood and mucus

- **amphipathic** a molecule containing both polar and nonpolar parts
- **amphitrichous** having two flagella or tufts of multiple flagella, with one flagellum or tuft located at each end of the bacterial cell
- **amphotericin B** antifungal drug of the polyene class that is used to treat several systemic fungal infections
- **amplitude** the height of a wave
- **anabolism** chemical reactions that convert simpler molecules into more complex ones
- **anaerobe chamber** closed compartment used to handle and grow obligate anaerobic cultures
- **anaerobe jar** container devoid of oxygen used to grow obligate anaerobes
- **anaerobic respiration** use of a non-oxygen inorganic molecule, like CO₂, nitrate, nitrite, oxidized iron, or sulfate, as the final electron acceptor at the end of the electron transport system
- **analytical epidemiology** study of disease outbreaks to establish associations between an agent and a disease state through observational studies comparing groups of individuals
- **anaphylactic shock** another term for anaphylaxis
- **anaphylaxis** systemic and potentially life-threatening type I hypersensitivity reaction
- **anergy** peripheral tolerance mechanism that prevents self-reactive T cells from being activated by self-antigens through lack of co-stimulation
- **annealing** formation of hydrogen bonds between the nucleotide base pairs of two single-stranded complementary nucleic acid sequences
- **anoxygenic photosynthesis** type of photosynthesis found in many photosynthetic bacteria, including the purple and green bacteria, where an electron donor other than H₂O is used to replace an electron lost by a reaction center pigment, resulting no oxygen production
- **anthrax** a disease caused by *Bacillus anthracis*; the cutaneous form causes a skin lesion to develop; gastrointestinal and inhalation anthrax have high mortality rates
- **antibiogram** compilation of the antimicrobial susceptibilities recorded for local bacterial strains, which is useful for monitoring local trends in antimicrobial resistance and aiding the prescription of appropriate empiric antibacterial therapy

- **antibiotic-associated diarrhea** diarrhea that develops after antibiotic treatment as a result of disruption to the normal microbiota; *C. difficile* is a particularly serious example
- **antibody screen** test to make sure that a potential blood recipient has not produced antibodies to antigens other than the ABO and Rh antigens
- **antibody** Y-shaped glycoprotein molecule produced by B cells that binds to specific epitopes on an antigen
- **antibody-dependent cell-mediated cytotoxicity (ADCC)** mechanism by which large pathogens are marked for destruction by specific antibodies and then killed by secretion of cytotoxins by natural killer cells, macrophages, or eosinophils
- **anticodon** three-nucleotide sequence of a mature tRNA that interacts with an mRNA codon through complementary base pairing
- **antigen (also, immunogen)** a molecule that stimulates an adaptive immune response
- **antigenic** able to stimulate an adaptive immune response
- **antigenic drift** form of slight antigenic variation that occurs because of point mutations in the genes that encode surface proteins
- **antigenic shift** form of major antigenic variation that occurs because of gene reassortment
- **antigenic variation** changing of surface antigens (carbohydrates or proteins) such that they are no longer recognized by the host's immune system
- **antigen-presenting cells (APC)** macrophages, dendritic cells, and B cells that process and present foreign pathogen antigens for the purpose of activating T cells and adaptive immune defenses
- **antimetabolites** compounds that are competitive inhibitors for bacterial metabolic enzymes
- **antimicrobial drugs** chemical compounds, including naturally produced drugs, semisynthetic derivatives, and synthetic compounds, that target specific microbial structures and enzymes, killing specific microbes or inhibiting their growth
- **antimicrobial peptides (AMPs)** class of nonspecific, cell-derived chemical mediators with broad-spectrum antimicrobial properties
- **antiparallel** two strands of DNA helix oriented in opposite directions; one strand is oriented in the 5' to 3' direction, while the other is

oriented in the 3' to 5' direction

- **antisense RNA** small noncoding RNA molecules that inhibit gene expression by binding to mRNA transcripts via complementary base pairing
- **antisense strand** transcription template strand of DNA; the strand that is transcribed for gene expression
- **antiseptis** protocol that removes potential pathogens from living tissue
- **antiseptic** antimicrobial chemical that can be used safely on living tissue
- **antiserum** serum obtained from an animal containing antibodies against a particular antigen that was artificially introduced to the animal
- **apoenzyme** enzyme without its cofactor or coenzyme
- **apoptosis** programmed and organized cell death without lysis of the cell
- **arachnoid mater** middle membrane surrounding the brain that produces cerebrospinal fluid
- **arboviral encephalitis** infection by an arthropod-borne virus that results in an inflammation of the brain
- **arbovirus** any of a variety of viruses that are transmitted by arthropod vectors
- **archaea** any of various unicellular prokaryotic microorganisms, typically having cell walls containing pseudopeptidoglycan
- **Archaea** domain of life separate from the domains Bacteria and Eukarya
- **artemisinin** antiprotozoan and antifungal drug effective against malaria that is thought to increase intracellular levels of reactive oxygen species in target microbes
- **artery** large, thick-walled vessel that carries blood from the heart to the body tissues
- **Arthus reaction** localized type III hypersensitivity
- **artificial active immunity** immunity acquired through exposure to pathogens and pathogen antigens through a method other than natural infection
- **artificial passive immunity** transfer of antibodies produced by a donor to another individual for the purpose of preventing or treating disease

- **ascariasis** soil-transmitted intestinal infection caused by the large nematode roundworm *Ascaris lumbricoides*
- **ascocarps** cup-shaped fruiting bodies of an ascomycete fungus
- **ascospore** asexual spore produced by ascomycete fungi
- **ascus** structure of ascomycete fungi containing spores
- **asepsis** sterile state resulting from proper use of microbial control protocols
- **aseptic technique** method or protocol designed to prevent microbial contamination of sterile objects, locations, or tissues
- **aspergillosis** fungal infection caused by the mold *Aspergillus*; immunocompromised patients are primarily at risk
- **asymptomatic carrier** an infected individual who exhibits no signs or symptoms of disease yet is capable of transmitting the pathogen to others
- **asymptomatic** not exhibiting any symptoms of disease
- **atomic force microscope** a scanning probe microscope that uses a thin probe that is passed just above the specimen to measure forces between the atoms and the probe
- **ATP synthase** integral membrane protein that harnesses the energy of the proton motive force by allowing hydrogen ions to diffuse down their electrochemical gradient, causing components of this protein to spin, making ATP from ADP and P_i
- **attachment** binding of phage or virus to host cell receptors
- **attenuation** regulatory system of prokaryotes whereby secondary stem-loop structures formed within the 5' end of an mRNA being transcribed determine both if transcription to complete the synthesis of this mRNA will occur and if this mRNA will be used for translation
- **autoclave** specialized device for the moist-heat sterilization of materials through the application of pressure to steam, allowing the steam to reach temperatures above the boiling point of water
- **autocrine function** refers to a cytokine signal released from a cell to a receptor on its own surface
- **autograft** tissue transplanted from a location on an individual to a different location on the same individual
- **autoimmune disease** loss of tolerance to self, resulting in immune-mediated destruction of self cells and tissues

- **autoinducer** signaling molecule produced by a bacterial cell that can modify the activity of surrounding cells; associated with quorum sensing
- **autoradiography** the method of producing a photographic image from radioactive decay; in molecular genetics the method allows the visualization of radioactively-labeled DNA probes that have hybridized to a nucleic acid sample
- **autotroph** organism that converts inorganic carbon dioxide into organic carbon
- **auxotroph** nutritional mutant with a loss-of-function mutation in a gene encoding the biosynthesis of a specific nutrient such as an amino acid
- **avidity** strength of the sum of the interactions between an antibody and antigen
- **axon** long projection of a neuron along which an electrochemical signal is transmitted
- **azithromycin** semisynthetic macrolide with increased spectrum of activity, decreased toxicity, and increased half-life compared with erythromycin

B

- **β -lactamases** bacterially produced enzymes that cleave the β -lactam ring of susceptible β -lactam antimicrobials, rendering them inactive and conferring resistance
- **β -lactams** group of antimicrobials that inhibit cell wall synthesis; includes the penicillins, cephalosporins, carbapenems, and monobactams; inhibits the transpeptidase cross-linking activity of penicillin-binding proteins
- **β -oxidation** process of fatty acid degradation that sequentially removes two-carbon acetyl groups, producing NADH and FADH₂, on entry into the Krebs cycle
- **β -pleated sheet** secondary structure consisting of pleats formed by hydrogen bonds between localized segments of amino acid residues on the backbone of the polypeptide chain

- **B-cell receptors (BCRs)** membrane-bound IgD and IgM antibody that bind specific antigen epitopes with Fab antigen-binding region
- **B lymphocyte** antibody-producing cells of humoral immunity; B cell
- **babesiosis** tickborne protozoan infection caused by *Babesia* spp. and characterized by malaise, fatigue, fever, headache, myalgia, and joint pain
- **bacillary dysentery** gastrointestinal illness caused by *Shigella* bacteria, also called shigellosis
- **bacillus** (bacilli) rod-shaped prokaryotic cell
- **bacitracin** group of structurally similar peptides that block the movement of peptidoglycan precursors across the cell membrane, inhibiting peptidoglycan synthesis
- **bacteremia** condition marked by the presence of bacteria in the blood
- **bacteria** (singular: bacterium) any of various unicellular prokaryotic microorganisms typically (but not always) having cell walls that contain peptidoglycan
- **bacterial lawn** layer of confluent bacterial growth on an agar plate
- **bacterial meningitis** bacterial infection that results in an inflammation of the meninges
- **bacterial vaginosis** a condition caused by an overgrowth of bacteria in the vagina that may or may not cause symptoms
- **bactericidal** irreversible inhibition of a microbe's ability to divide
- **bactericide** chemical or physical treatment that kills bacteria
- **bacteriochlorophylls** green, purple, or blue pigments of bacteria; they are similar to chlorophyll of plants
- **bacteriology** the study of bacteria
- **bacteriophage** virus that infects bacteria
- **bacteriostatic** having the ability to inhibit bacterial growth, generally by means of chemical or physical treatment; reversible inhibition of a microbe's ability to divide
- **barophile** organism that grows under high atmospheric pressure
- **basal body** component of eukaryotic flagellum or cilium composed of nine microtubule triplets and attaches the flagellum or cilium to the cell
- **base sequence** identity of the specific nucleotides present in a nucleic acid strand and their order within the strand

- **basic dye** a chromophore with a positive charge that attaches to negatively charged structures
- **basidia (basidium, sing.)** small club-shaped structures of basidiomycete fungi where basidiospores are produced
- **basidiocarps** fruiting bodies of basidiomycete fungi
- **basidiospores** spores produced sexually via budding in basidiomycete fungi
- **basophils** leukocytes with granules containing histamine and other chemicals that facilitate allergic responses and inflammation when released
- **benzimidazoles** class of antihelminthic drugs that bind to helminthic β -tubulin, preventing microtubule formation
- **Betaproteobacteria** class of Proteobacteria that are all eutrophs
- **binary fission** predominant form of bacterial reproduction in which one cell divides into two daughter cells of equal size, which separate, each offspring receiving a complete copy of the parental genome
- **binocular** having two eyepieces
- **binomial nomenclature** a universal convention for the scientific naming of organisms using Latinized names for genus and species
- **biofilm** complex ecosystem of bacteria embedded in a matrix
- **biogeochemical cycle** recycling of inorganic matter between living organisms and their nonliving environment
- **bioinformatics** the analysis of large amounts of information required for interpretation of these data
- **biological transmission** movement of a pathogen between hosts facilitated by a biological vector in which the pathogen grows and reproduces
- **biological vector** an animal (typically an arthropod) that is infected with a pathogen and is capable of transmitting the pathogen from one host to another
- **biomarker** a protein expressed by a cell or tissue that is indicative of disease
- **biomolecule** a molecule that is part of living matter
- **bioremediation** use of microbes to remove xenobiotics or environmental pollutants from a contaminated site
- **biosynthesis** replication of viral genome and other protein components

- **biotechnology** the science of using living systems to benefit humankind
- **bisbiguanide** type of chemical compound with antiseptic properties; disrupts cell membranes at low concentrations and causes congealing of intracellular contents at high concentrations
- **blastomycosis** fungal disease associated with infections by *Blastomyces dermatitidis*; can cause disfiguring scarring of the hands and other extremities
- **blepharitis** inflammation of the eyelids
- **blocking antibodies** antigen-specific antibodies (usually of the IgG type) produced via desensitization therapy
- **blood-brain barrier** tight cell junctions of the endothelia lining the blood vessels that serve the central nervous system, preventing passage of microbes from the bloodstream into the brain and cerebrospinal fluid
- **blue-white screening** a technique commonly used for identifying transformed bacterial cells containing recombinant plasmids using *lacZ*-encoding plasmid vectors
- **blunt ends** ends of DNA molecules lacking single-stranded complementary overhangs that are produced when some restriction enzymes cut DNA
- **botulism** form of flaccid paralysis caused by the ingestion of a neurotoxin produced by *Clostridium botulinum*
- **bradykinin** activated form of a proinflammatory molecule induced in the presence of invader microbes; opens gaps between cells in blood vessels, allowing fluid and cells to leak into surrounding tissue
- **bridge reaction** reaction linking glycolysis to the Krebs cycle during which each pyruvate is decarboxylated and oxidized (forming NADH), and the resulting two-carbon acetyl group is attached to a large carrier called coenzyme A, resulting in the formation of acetyl-CoA and CO₂; also called the *transition reaction*
- **brightfield microscope** a compound light microscope with two lenses; it produces a dark image on a bright background
- **broad-spectrum antimicrobial** drug that targets many different types of microbes
- **bronchi** major air passages leading to the lungs after bifurcating at the windpipe

- **bronchioles** smaller air passages within the lung that are formed as the bronchi become further subdivided
- **bronchitis** inflammation of the bronchi
- **brucellosis** zoonotic disease caused by bacteria of the genus *Brucella* that results in undulant fever
- **bubo** swollen, inflamed lymph node that forms as a result of a microbial infection
- **bubonic plague** most common form of plague in humans, marked by the presence of swollen lymph nodes (buboes)
- **budding** unequal reproductive division in which a smaller cell detaches from the parent cell
- **budding yeasts** yeasts that divide by budding off of daughter cells
- **Burkitt lymphoma** disease characterized by rapidly growing solid tumor; caused by Epstein-Barr virus (HHV-4)
- **burst** release of new virions by a lysed host cell infected by a virus
- **burst size** the number of virions released from a host cell when it is lysed because of a viral infection

C

- **Calvin-Benson cycle** most common CO₂ fixation pathway in most photoautotrophs; involves light-independent reactions of photosynthesis that occur in the cytoplasm of photosynthetic bacteria and in the stroma of eukaryotic chloroplasts
- ***Campylobacter jejuni* gastroenteritis** gastroenteritis caused by *C. jejuni*; generally mild but sometimes with serious complications
- **candidiasis** fungal infection caused by *Candida* spp., especially *C. albicans*; can affect various regions of the body, e.g., skin (cutaneous candidiasis), oral cavity (oral thrush), or vagina (yeast infection)
- **candle jar** container with a tight-fitting lid in which a burning candle consumes oxygen and releases carbon dioxide, thereby creating an environment suitable for capnophiles
- **capillary** small blood vessel found in the interstitial space of tissue; delivers nutrients and oxygen, and removes waste products
- **capnophile** organism that requires carbon dioxide levels higher than atmospheric concentration

- **capsid** protein coat surrounding the genome of the virus
- **capsomere** individual protein subunits that make up the capsid
- **capsule staining** a negative staining technique that stains around a bacterial capsule while leaving the capsule clear
- **capsule** type of glycocalyx with organized layers of polysaccharides that aid in bacterial adherence to surfaces and in evading destruction by immune cells
- **carbapenem-resistant Enterobacteriaceae (CRE)** group of bacteria that have developed resistance to all β -lactams, including carbapenems, and many other drug classes
- **carbohydrate** the most abundant type of biomolecule, consisting of carbon, hydrogen, and oxygen
- **carbon skeleton** chain of carbon atoms to which one or more functional groups are bound
- **carboxysome** an inclusion composed of an outer shell of thousands of protein subunits. Its interior is filled with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase, which are both used for carbon metabolism
- **carbuncle** abscess containing a large, deep, purulent skin lesion
- **carcinogen** agent that causes cancer
- **case-control study** a type of observational study in which a group of affected individuals are compared, usually retrospectively, to a similar group of unaffected individuals
- **catabolic activator protein (CAP)/cAMP receptor protein (CRP)** protein that, when bound to cAMP in the presence of low levels of glucose, binds to the promoters of operons that control the processing of alternative sugars
- **catabolism** chemical reactions that break down complex molecules into simpler ones
- **catabolite repression** repression of the transcription of operons encoding enzymes for the use of substrates other than glucose when glucose levels are high
- **catalase** enzyme that breaks down hydrogen peroxide to water and oxygen
- **catalyst** molecule that increases the rate of a chemical reaction but is not used or changed during the chemical reaction and, thus, is reusable

- **catarrhal stage** in pertussis, a disease stage marked by inflammation of the mucous membranes combined with excessive secretions
- **cat-scratch disease** bacterial infection of the lymph nodes caused by *Bartonella henselae*; frequently transmitted via a cat scratch
- **causative agent** the pathogen or substance responsible for causing a particular disease; etiologic agent
- **CCA amino acid binding end** region of a mature tRNA that binds to an amino acid
- **celiac disease** disease largely of the small intestine caused by an immune response to gluten that results in the production of autoantibodies and an inflammatory response
- **cell envelope** the combination of external cellular structures (e.g., plasma membrane, cell wall, outer membrane, glycocalyxes) that collectively contain the cytoplasm and internal structures of a cell
- **cell membrane** lipid bilayer with embedded proteins and carbohydrates that defines the boundary of the cell (also called the cytoplasmic membrane or plasma membrane)
- **cell morphology** cell shape, structure, and arrangement, as viewed microscopically
- **cell theory** the theory that all organisms are composed of cells and that the cell is the fundamental unit of life
- **cell wall** a structure in the cell envelope of some cells that helps the cell maintain its shape and withstand changes in osmotic pressure
- **cellular immunity** adaptive immunity involving T cells and the destruction of pathogens and infected cells
- **cellulitis** a subcutaneous skin infection that develops in the dermis or hypodermis, resulting in a red, painful inflammation
- **cellulose** a structural polysaccharide composed of glucose monomers linked together in a linear chain by glycosidic bonds
- **Centers for Disease Control and Prevention (CDC)** the national public health agency in the United States
- **central dogma** scientific principle explaining the flow of genetic information from DNA to RNA to protein
- **central nervous system (CNS)** portion of the nervous system made up of the brain and spinal cord
- **central tolerance** negative selection of self-reactive T cells in thymus

- **centriole** a component of a centrosome with the structural array of nine parallel microtubules arranged in triplets; involved in eukaryotic cell division
- **centrosome** a microtubule-organizing center for the mitotic spindle found in animal cells; it separates chromosomes during cell division and is composed of a pair of centrioles positioned at right angles to each other
- **cephalosporins** a group of cell wall synthesis inhibitors within the class of β -lactams
- **cercarial dermatitis** inflammation of the skin caused by a reaction to cercaria of *Schistosoma* spp., which can penetrate the skin and blood vessels; also called swimmer's itch or clam digger's itch
- **cerebrospinal fluid (CSF)** sterile liquid produced in the brain that fills the subarachnoid space of the brain and spinal column
- **cervix** the part of the uterus that connects to the vagina
- **CFB group** phylum consisting of the gram-negative, rod-shaped nonproteobacteria genera *Cytophaga*, *Fusobacterium*, and *Bacteroides*
- **Chagas disease** potentially fatal protozoan infection caused by *Trypanosoma cruzi* and endemic to Central and South America; transmitted by the triatomine bug (kissing bug)
- **chancroid** an STI caused by *Haemophilus ducreyi* that produces soft chancres on genitals
- **charged tRNA** activated tRNA molecule carrying its cognate amino acid
- **chemical mediators** chemicals or enzymes produced by a variety of cells; provide nonspecific antimicrobial defense mechanisms
- **chemically defined media** media in which all components are chemically defined
- **chemiosmosis** flow of hydrogen ions across the membrane through ATP synthase
- **chemokines** chemotactic cytokines that recruit specific subsets of leukocytes to infections, damaged tissue, and sites of inflammation
- **chemotaxis** directional movement of a cell in response to a chemical attractant
- **chemotroph** organism that gets its energy from the transfer of electrons originating from chemical compounds

- **chickenpox** common childhood disease caused by the varicella-zoster virus and marked by the formation of pustular lesions on the trunk
- **chikungunya fever** mosquito-borne viral disease caused by the chikungunya virus and characterized by high fever, joint pain, rash, and blisters
- **chirality** property of stereoisomer molecules by which their structures are nonsuperimposable mirror-images
- **chitin** polysaccharide that is an important component of fungal cell walls
- **chlamydia** a common STI caused by *Chlamydia trachomatis*
- **chloramphenicol** protein synthesis inhibitor with broad-spectrum activity that binds to the 50S subunit, inhibiting peptide bond formation
- **chlorophyll** a type of photosynthetic pigment found in some prokaryotic and eukaryotic cells
- **chloroplast** organelle found in plant and algal cells in which photosynthesis occurs
- **cholera** gastrointestinal illness caused by *Vibrio cholera* characterized by severe diarrhea
- **chromatin** combination of DNA with DNA binding proteins
- **chromogenic substrate** colorless substrate (chromogen) that is converted into a colored end product by the enzyme
- **chromophores** pigments that absorb and reflect particular wavelengths of light (giving them a color)
- **chromosome** discrete DNA structure within a cell that controls cellular activities
- **chronic disease** any disease that progresses and persists over a long time
- **chronic granulomatous disease** primary immunodeficiency caused by an impaired ability of phagocytic cells to kill ingested bacteria in the phagolysosome
- **chronic wasting disease** prion disease of deer and elk in the United States and Canada
- **cilia (singular: cilium)** short filamentous structures found on some eukaryotic cells; each is composed of microtubules in a 9+2 array, and may be used for locomotion, feeding, and/or movement of extracellular particles that come in contact with the cell

- **ciliated epithelial cells** hair-like cells in the respiratory tract that beat, pushing mucus secretions and trapped debris away from the sensitive tissues of the lungs
- **ciliates** protists with cilia (Ciliophora), including *Paramecium* and *Stentor*, classified within the Chromalveolata
- **cisternae** the sacs of the endoplasmic reticulum
- **citric acid cycle** see *Krebs cycle*
- **class switching** genetic rearrangement of constant region gene segments in plasma cells to switch antibody production from IgM to IgG, IgA, or IgE
- **clindamycin** semisynthetic protein synthesis inhibitor of the lincosamide class that binds to the 50S subunit, inhibiting peptide bond formation
- **clone** a genetically identical cell or individual
- ***Clostridium perfringens* gastroenteritis** relatively mild gastrointestinal illness caused by *C. perfringens*
- **clusters of differentiation (CD)** cell-surface glycoproteins that serve to identify and distinguish white blood cells
- **coagulase** enzyme that causes the activation of fibrinogen to form fibrin, promoting clotting of the blood
- **coarse focusing knob** a knob on a microscope that produces relatively large movements to adjust focus
- **coccidioidomycosis** disease caused by the highly infectious fungal pathogen *Coccidioides immitis* and related species
- **codon** three-nucleotide sequence within mRNA that specifies a particular amino acid to be incorporated into the polypeptide being synthesized
- **coenocyte** multinucleated eukaryotic cell that forms as a result of multiple rounds of nuclear division without the accompanying division of the plasma membrane
- **coenocytic hyphae** nonseptate hyphae that are multinucleate and lack cell walls or membranes between cells; characteristic of some fungi
- **coenzyme** organic molecule required for proper enzyme function that is not consumed and is reusable
- **cofactor** inorganic ion that helps stabilize enzyme conformation and function

- **cognate amino acid** amino acid added to a specific tRNA molecule that correctly corresponds to the tRNA's anticodon and, hence, the mRNA's codon, reflecting the genetic code
- **cohort method** a method used in observational studies in which a group of individuals is followed over time and factors potentially important in the development of disease are evaluated
- **colistin** membrane-active polymyxin that was historically used for bowel decontamination but now used for systemic infections with drug-resistant pathogens
- **colitis** inflammation of the large intestine
- **collagenase** enzyme that digests collagen, the dominant protein in connective tissue
- **colony-forming unit (CFU)** a counting quantity represented by a colony formed on solid medium from a single cell or a few cells
- **commensalism** type of symbiosis in which one population benefits and the other is not affected
- **commercial sterilization** type of sterilization protocol used in food production; uses conditions that are less harsh (lower temperatures) to preserve food quality but still effectively destroy vegetative cells and endospores of common foodborne pathogens such as *Clostridium botulinum*
- **common cold** most common cause of rhinitis in humans; associated with a variety of adenoviruses, coronaviruses, and rhinoviruses
- **common source spread** a mode of disease transmission in which every infection originates from the same source
- **communicable** able to be transmitted directly or indirectly from one person to another
- **community** group of interacting populations of organisms
- **competitive inhibitor** molecule that binds to an enzyme's active site, preventing substrate binding
- **competitive interactions** interactions between populations in which one of them competes with another for resources
- **complement activation** cascading activation of the complement proteins in the blood, resulting in opsonization, inflammation, and lysis of pathogens
- **complement fixation test** test for antibodies against a specific pathogen using complement-mediated hemolysis

- **complement system** series of proteins that can become activated in the presence of invading microbes, resulting in opsonization, inflammation, and lysis of pathogens
- **complementary base pairs** base pairing due to hydrogen bonding that occurs between a specific purine and a specific pyrimidine; A bonds with T (in DNA), and C bonds with G
- **complementary DNA (cDNA)** a DNA molecule complementary to mRNA that is made through the activity of reverse transcriptase
- **complex media** media that contain extracts of animals and plants that are not chemically defined
- **complex virus** virus shape that often includes intricate characteristics not seen in the other categories of capsid
- **compound microscope** a microscope that uses multiple lenses to focus light from the specimen
- **condenser lens** a lens on a microscope that focuses light from the light source onto the specimen
- **conditional mutation** mutant form of a gene whose mutant phenotype is expressed only under certain environmental conditions
- **confocal microscope** a scanning laser microscope that uses fluorescent dyes and excitation lasers to create three-dimensional images
- **conidia** asexual fungal spores not enclosed in a sac; produced in a chain at the end of specialized hyphae called conidiophores
- **conjugate vaccine** a vaccine consisting of a polysaccharide antigen conjugated to a protein to enhance immune response to the polysaccharide; conjugate vaccines are important for young children who do not respond well to polysaccharide antigens
- **conjugated protein** protein carrying a nonpolypeptidic portion
- **conjugation** mechanism of horizontal gene transfer in bacteria in which DNA is directly transferred from one bacterial cell to another by a conjugation pilus
- **conjugation pilus (sex pilus)** hollow tube composed of protein encoded by the conjugation plasmid that brings two bacterial cells into contact with each other for the process of conjugation
- **conjunctiva** the mucous membranes covering the eyeball and inner eyelid
- **conjunctivitis** inflammation of the conjunctiva, the mucous membrane covering the eye and inside of the eyelid

- **constitutively expressed** describes genes that are transcribed and translated continuously to provide the cell with constant intermediate levels of the protein products
- **contact dermatitis** inflammation of the skin resulting from a type IV hypersensitivity to an allergen or irritant
- **contact** see *exposure*
- **contact transmission** movement of a pathogen between hosts due to contact between the two; may be direct or indirect
- **contagious** easily spread from person to person
- **continuous cell line** derived from transformed cells or tumors, these cells are often able to be subcultured many times, or, in the case of immortal cell lines, grown indefinitely
- **continuous common source spread** a mode of disease transmission in which every infection originates from the same source and that source produces infections for longer than one incubation period
- **contractile vacuoles** organelles found in some cells, especially in some protists, that take up water and then move the water out of the cell for osmoregulatory purposes (i.e., to maintain an appropriate salt and water balance)
- **contrast** visible differences between parts of a microscopic specimen
- **convalescence stage** the final stage of a whooping cough infection, marked by a chronic cough
- **Coombs' reagent** antiserum containing antihuman immunoglobulins used to facilitate hemagglutination by cross-linking the human antibodies attached to red blood cells
- **cooperative interactions** interactions between populations in which both benefit
- **cortex** tightly packed layer of fungal filaments at the outer surface of a lichen; foliose lichens have a second cortex layer beneath the medulla
- **counterstain** a secondary stain that adds contrasting color to cells from which the primary stain has been washed out by a decolorizing agent
- **crenation** shriveling of a cell
- **Creutzfeldt-Jakob disease** form of transmissible spongiform encephalopathy found in humans; typically a fatal disease
- **crisis phase** point at which a fever breaks, reaching a peak before the hypothalamus resets back to normal body temperature

- **critical item** object that must be sterile because it will be used inside the body, often penetrating sterile tissues or the bloodstream
- **cross-match** in the major cross-match, donor red blood cells are checked for agglutination using recipient serum; in the minor cross-match, donor serum is checked for agglutinating antibodies against recipient red blood cells
- **cross-presentation** a mechanism by which dendritic cells process antigens for MHC I presentation to CD8 T cells through phagocytosis of the pathogen (which would normally lead to MHC II presentation)
- **cross-resistance** when a single resistance mechanism confers resistance to multiple antimicrobial drugs
- **cross-sectional study** a type of observational study in which measurements are made on cases, both affected and unaffected, at one point in time and the measurements analyzed to uncover associations with the disease state
- **crustose lichens** lichens that are tightly attached to the substrate, giving them a crusty appearance
- **cryptococcosis** fungal pneumonia caused by the encapsulated yeast *Cryptococcus neoformans* commonly found in bird droppings
- **cryptosporidiosis** intestinal infection caused by *Cryptosporidium parvum* or *C. hominis*
- **culture density** the number of cells per volume of broth
- **culture medium** combination of compounds in solution that supports growth
- **cutaneous mycosis** any fungal infection that affects the surface of the skin, hair, or nails
- **cyanobacteria** phototrophic, chlorophyll-containing bacteria that produce large amounts of gaseous oxygen
- **cyclic AMP (cAMP)** intracellular signaling molecule made through the action of adenylyl cyclase from ATP when glucose levels are low, with the ability to bind to a catabolite activator protein to allow it to bind to regulatory regions and activate the transcription of operons encoding enzymes for metabolism of alternative substrates
- **cyclic photophosphorylation** pathway used in photosynthetic organisms when the cell's need for ATP outweighs that for NADPH, thus bypassing NADPH production
- **cyclosporiasis** intestinal infection caused by *Cyclospora cayetanensis*

- **cystic echinococcosis** hydatid disease, an infection caused by the tapeworm *Echinococcus granulosus* that can cause cyst formation
- **cysticerci** larval form of a tapeworm
- **cystitis** inflammation of the bladder
- **cysts** microbial cells surrounded by a protective outer covering; some microbial cysts are formed to help the microbe survive harsh conditions, whereas others are a normal part of the life cycle
- **cytochrome oxidase** final ETS complex used in aerobic respiration that transfers energy-depleted electrons to oxygen to form H₂O
- **cytokine storm** an excessive release of cytokines, typically triggered by a superantigen, that results in unregulated activation of T cells
- **cytokines** protein molecules that act as a chemical signals; produced by cells in response to a stimulation event
- **cytomegalovirus (CMV) infection** human herpesvirus 5 infection that is typically asymptomatic but can become serious in immunocompromised patients, transplant recipients, and developing fetuses
- **cytopathic effect** cell abnormality resulting from a viral infection
- **cytoplasm** the gel-like material composed of water and dissolved or suspended chemicals contained within the plasma membrane of a cell
- **cytoplasmic membrane** see *cell membrane*
- **cytoproct** a protozoan cell structure that is specialized for excretion
- **cytosine** pyrimidine nitrogenous base found in nucleotides
- **cytoskeleton** a network of filaments or tubules in the eukaryotic cell that provides shape and structural support for cells; aids movement of materials throughout the cell
- **cytostome** a protozoan cell structure that is specialized for phagocytosis (i.e., to take in food)
- **cytotoxic T cells** effector cells of cellular immunity that target and eliminate cells infected with intracellular pathogens through induction of apoptosis
- **cytotoxicity** harmful effects to host cell

- **dacryocystitis** inflammation of the lacrimal sac often associated with a plugged nasolacrimal duct
- **daptomycin** cyclic lipopeptide that disrupts the bacterial cell membrane
- **darkfield microscope** a compound light microscope that produces a bright image on a dark background; typically a modified brightfield microscope
- **death phase (decline phase)** phase of the growth curve at which the number of dying cells exceeds the number of new cells formed
- **decimal reduction time (DRT) or D-value** amount of time it takes for a specific protocol to produce a one order of magnitude decrease in the number of organisms; that is, death of 90% of the population
- **decolorizing agent** a substance that removes a stain, usually from some parts of the specimen
- **deeply branching bacteria** bacteria that occupy the lowest branches of the phylogenetic tree of life
- **definitive host** the preferred host organism for a parasite, in which the parasite reaches maturity and may reproduce sexually
- **degeneracy** redundancy in the genetic code because a given amino acid is encoded by more than one nucleotide triplet codon
- **degerming** protocol that significantly reduces microbial numbers by using mild chemicals (e.g., soap) and gentle scrubbing of a small area of skin or tissue to avoid the transmission of pathogenic microbes
- **degranulation** release of the contents of mast cell granules in response to the cross-linking of IgE molecules on the cell surface with allergen molecules
- **dehydration synthesis** chemical reaction in which monomer molecules bind end to end in a process that results in the formation of water molecules as a byproduct
- **deletion** type of mutation involving the removal of one or more bases from a DNA sequence
- **Deltaproteobacteria** class of Proteobacteria that includes sulfate-reducing bacteria
- **denatured protein** protein that has lost its secondary and tertiary structures (and quaternary structure, if applicable) without the loss of its primary structure
- **dendrites** branched extensions of the soma of a neuron that interact with other cells

- **dengue fever** mosquito-borne viral hemorrhagic disease; also known as breakbone fever
- **dental calculus** calcified heavy plaque on teeth, also called tartar
- **dental caries** cavities formed in the teeth as a result of tooth decay caused by microbial activity
- **deoxyribonucleic acid (DNA)** double-stranded nucleic acid composed of deoxyribonucleotides that serves as the genetic material of the cell
- **deoxyribonucleotides** DNA nucleotides containing deoxyribose as the pentose sugar component
- **dermatophyte** any fungus of the genera *Microsporum*, *Epidermophyton*, or *Trichophyton*, which feed on keratin (a protein found in skin, hair, and nails) and can cause cutaneous infections
- **dermis** the second layer of human skin, found between the epidermis and the hypodermis
- **descriptive epidemiology** a method of studying a disease outbreak using case histories, contact interviews, medical information, and other sources of information
- **desensitization** injections of antigen that lead to production of antigen-specific IgG molecules, effectively outcompeting IgE molecules on the surface of sensitized mast cells for antigen
- **desiccation** method of microbial control involving the removal of water from cells through drying or dehydration
- **desquamation** peeling and shedding of outermost skin
- **diapedesis** process by which leukocytes pass through capillary walls to reach infected tissue; also called extravasation
- **diaphragm** a component of a microscope; typically consists of a disk under the stage with holes of various sizes; can be adjusted to allow more or less light from the light source to reach the specimen
- **differential interference-contrast microscope** a microscope that uses polarized light to increase contrast
- **differential media** media that contain additives that make it possible to distinguish bacterial colonies based on metabolic activities of the organisms
- **differential staining** staining that uses multiple dyes to differentiate between structures or organisms
- **diffraction** the changing of direction (bending or spreading) that occurs when a light wave interacts with an opening or barrier

- **dikaryotic** having two separate nuclei within one cell
- **dimorphic fungus** a fungus that can take the form of a yeast or a mold, depending on environmental conditions
- **dioecious** refers to sexually reproducing organisms in which individuals have either male or female reproductive organs (not both)
- **diphtheria** serious infection of the larynx, caused by the toxigenic bacterium *Corynebacterium diphtheriae*
- **diploid** having two copies of each chromosome
- **direct agglutination assay** assay that can be used to detect the agglutination of bacteria by the action of antibodies in patient serum
- **direct antihuman globulin test (DAT)** another name for a direct Coombs' test
- **direct contact transmission** movement of a pathogen between hosts by physical contact or transfer in droplets at a distance less than one meter
- **direct Coombs' test** assay that looks for antibodies *in vivo* against red blood cells caused by various types of infections, drug reactions, and autoimmune disorders
- **direct ELISA** enzyme-linked immunoabsorbent assay in which the antigens are immobilized in the well of a microtiter plate; only a single antibody is used in the test
- **direct fluorescent antibody (DFA) test** FA technique in which the labeled antibody binds to the target antigen
- **direct hemagglutination assay** test that determines the titer of certain bacteria and viruses that causes clumping of red blood cells
- **direct microscopic cell count** counting of cells using a calibrated slide under a light microscope
- **direct repair (light repair or photoreactivation)** light-dependent mechanism for repairing pyrimidine dimers involving the enzyme photolyase
- **disaccharide** one of two monosaccharides linked together by a glycosidic bond
- **disease** any condition in which the normal structure or function of the body is damaged or impaired
- **disinfectant** antimicrobial chemical applied to a fomite during disinfection that may be toxic to tissues
- **disinfection** protocol that removes potential pathogens from a fomite

- **disk-diffusion method** a technique for measuring of the effectiveness of one or more antimicrobial agents against a known bacterium; involves measuring the zone(s) of inhibition around the chemical agent(s) in a culture of the bacterium
- **dispersion** the separation of light of different frequencies due to different degrees of refraction
- **disulfide bridge** covalent bond between the sulfur atoms of two sulfhydryl side chains
- **DNA gyrase (topoisomerase II)** bacterial topoisomerase that relaxes the supercoiled chromosome to make DNA more accessible for the initiation of replication
- **DNA ligase** enzyme that catalyzes the formation of a covalent phosphodiester linkage between the 3'-OH end of one DNA fragment and the 5' phosphate end of another DNA fragment
- **DNA packaging** process in which histones or other DNA binding proteins perform various levels of DNA wrapping and attachment to scaffolding proteins to allow the DNA to fit inside a cell
- **DNA polymerase** class of enzymes that adds nucleotides to the free 3'-OH group of a growing DNA chain that are complementary to the template strand
- **DNA primers** short, synthetic, single-stranded DNA fragments of known sequence that bind to specific target sequences within a sample due to complementarity between the target DNA sequence and the primer; commonly used in PCR but may be used in other hybridization techniques
- **DNA probe** a single-stranded DNA fragment that is complementary to part of the gene (DNA or RNA) of interest
- **DNase** pathogen-produced nuclease that degrades extracellular DNA
- **dosage** amount of medication given during a certain time interval
- **double immunodiffusion** see Ouchterlony assay
- **doubling time** the time it takes for the population to double; also referred to as generation time
- **droplet transmission** direct contact transmission of a pathogen transferred in sneezed or coughed droplets of mucus that land on the new host within a radius of one meter
- **drug resistance** ability of a microbe to persist and grow in the presence of an antimicrobial drug

- **dry-heat sterilization** protocol that involves the direct application of high heat
- **dura mater** tough, outermost membrane that surrounds the brain
- **dynein** motor proteins that interact with microtubules in eukaryotic flagella and cilia
- **dysentery** intestinal inflammation that causes diarrhea with blood and mucus
- **dysuria** urination accompanied by burning, discomfort, or pain

E

- **E (exit) site** functional site of an intact ribosome that releases dissociated uncharged tRNAs so that they can be recharged with free amino acids
- **East African trypanosomiasis** acute form of African trypanosomiasis caused by *Trypanosoma brucei rhodesiense*
- **eastern equine encephalitis** serious, but rare, mosquito-borne viral infection of the brain that is found primarily on the Atlantic and Gulf coast states of the United States
- **Ebola virus disease (EVD)** potentially fatal viral hemorrhagic fever found primarily in western Africa and transmitted through contact with body fluids
- **eclipse phase** period after viral infection during which the infective virus is not detected, either intracellularly or extracellularly, and biosynthesis is occurring
- **ectoplasm** outer, more gelatinous layer of cytoplasm under a protist cell membrane
- **edema** swelling due to accumulation of fluid and protein in tissue as a result of increased permeability of capillary walls during an inflammatory response; chronic edema can also result from blockage of lymphatic vessels, as in the case of elephantiasis
- **effector cells** activated cells of cellular immunity that are involved in the immediate immune response, primarily to defend the body against pathogens
- **electron carrier** cellular molecule that accepts high-energy electrons from reduced molecules like foods and later serves as an electron

donor in subsequent redox reactions

- **electron microscope** a type of microscope that uses short-wavelength electron beams rather than light to increase magnification and resolution
- **electron transport system (ETS)** series of membrane-associated protein complexes and associated mobile accessory electron carriers important in the generation of the proton motive force required for ATP production by chemiosmosis; the last component involved in the cellular respiration of glucose
- **electroporation** a genetic engineering technique in which cells are exposed to a short electric pulse, inducing them to take up DNA molecules from their environment
- **elementary bodies** metabolically and reproductively inactive, endospore-like form of intracellular bacteria that spreads infection outside of cells
- **elongation in DNA replication** stage of DNA replication during which DNA polymerase adds nucleotides, complementary to the parental strand, to the 3' end of a growing DNA strand
- **elongation in transcription** stage of transcription during which RNA polymerase extends the RNA molecule by adding RNA nucleotides, complementary to the template DNA strand
- **elongation of translation** stage of translation during which amino acids are added one by one to the C-terminus of the growing polypeptide
- **Embden-Meyerhof-Parnas (EMP) pathway** type of glycolysis found in animals and the most common in microbes
- **emerging infectious disease** a disease that is new to the human population or has increased in prevalence over the previous 20 years
- **enantiomers** stereoisomers that are mirror images of each other and nonsuperimposable
- **encephalitis** inflammation of the tissues of the brain
- **encystment** the process of forming a cyst
- **endemic disease** an illness that is constantly present (often at low levels) in a population
- **endergonic reaction** chemical reaction that requires energy beyond activation energy to occur

- **endocarditis** inflammation of the endocardium, especially the heart valves
- **endocrine function** refers to a cytokine signal released from a cell and carried by the bloodstream to a distant recipient cell
- **endocytosis** the uptake of molecules through plasma membrane invagination and vacuole/vesicle formation
- **endomembrane system** a series of organelles (endoplasmic reticulum, Golgi apparatuses, lysosomes, and transport vesicles) arranged as membranous tubules, sacs, and disks that synthesize many cell components
- **endoplasm** inner, more fluid layer of cytoplasm under a protist cell membrane (inside of the ectoplasm)
- **endoplasmic reticulum** part of the endomembrane system that is an interconnected array of tubules and flattened sacs with a single lipid bilayer that may be either rough or smooth; important in synthesizing proteins and lipids
- **endospore** a cellular structure formed by some bacteria in response to adverse conditions; preserves DNA of the cell in a dormant state until conditions are favorable again
- **endospore staining** a differential staining technique that uses two stains to make bacterial endospores appear distinct from the rest of the cell
- **endosymbiotic theory** the theory that mitochondria and chloroplasts arose as a result of prokaryotic cells establishing a symbiotic relationship within a eukaryotic host
- **endothelia** layer of epithelial cells lining blood vessels, lymphatics, the blood-brain barrier, and some other tissues
- **endotoxin** lipid A component of lipopolysaccharides in the outer membrane of gram-negative bacteria
- **enriched media** media that contain additional essential nutrients to support growth
- **enrichment culture** media providing growth conditions that favor the expansion of an organism present in low numbers
- **enteric** bacteria of the family Enterobacteriaceae, which live in the human intestinal tract
- **enteritis** inflammation of the lining of the intestine

- **enterobiasis** intestinal infection caused by the pinworm *Enterobius vermicularis*
- **enterohemorrhagic *E. coli* (EHEC)** *E. coli* bacteria that cause severe gastrointestinal illness with potential serious complications such as hemolytic uremic syndrome
- **enteroinvasive *E. coli* (EIEC)** *E. coli* bacteria that cause relatively mild gastrointestinal illness
- **enteropathogenic *E. coli* (EPEC)** *E. coli* bacteria that cause serious gastrointestinal illness
- **enterotoxigenic *E. coli* (ETEC)** *E. coli* bacteria that cause a relatively mild illness commonly called traveler's diarrhea
- **enterotoxin** toxin that affects the intestines
- **Entner-Doudoroff (ED) pathway** alternative glycolytic pathway used by some bacteria
- **enveloped virus** a virus formed with a nucleic-acid packed capsid surrounded by a lipid layer
- **enzyme** catalyst for biochemical reactions inside cells
- **enzyme immunoassay (EIA)** type of assay wherein an enzyme is coupled to an antibody; addition of a chromogenic substrate for the antibody allows quantification or identification of the antigen bound by the antibody
- **enzyme-linked immunosorbent assay (ELISA)** specialized form of EIA in which either the primary antibody or the antigen is first attached to a solid surface such as the well of a microtiter plate
- **eosinophils** leukocytes with granules containing histamine and major basic protein; facilitate allergic responses and protection against parasitic protozoa and helminths
- **epidemic disease** an illness with a higher-than-expected incidence in a given period within a given population
- **epidemic typhus** severe and sometimes fatal infection caused by *Rickettsia prowazekii* and transmitted by body lice
- **epidemiology** the study of where and when infectious diseases occur in a population and how they are transmitted and maintained in nature
- **epidermis** the outermost layer of human skin
- **epididymis** coiled tube that collects sperm from the testes and passes it on to the vas deferens

- **epididymitis** inflammation of the epididymis caused by a bacterial infection
- **epigenetic regulation** chemical modification of DNA or associated histones to influence transcription
- **epiglottis** flap of cartilage that covers the larynx during swallowing; diverts food to the esophagus and prevents it from entering the respiratory tract
- **epiglottitis** inflammation of the epiglottis
- **epiphyte** a plant that grows on another plant
- **epitope** smaller exposed region on an antigen that is recognized by B-cell and T-cell receptors and antibodies
- **Epsilonproteobacteria** class of Proteobacteria that are microaerophilic
- **equivalence zone** region where the antibody–antigen ratio produces the greatest amount of precipitin in a precipitin reaction
- **erysipelas** a skin infection, typically caused by *Streptococcus pyogenes*, that presents as a red, large, intensely inflamed patch of skin involving the dermis, usually with clear borders, typically on the legs or face
- **erythema nodosum** a condition that causes inflammation in the subcutaneous fat cells of the hypodermis resulting in red nodules
- **erythema** redness at the site of inflammation, usually due to dilation of blood vessels in the area to help bring in white blood cells
- **erythrocyte** red blood cell
- **erythrogenic toxin** exotoxin produced by some strains of *Streptococcus pyogenes*; activity of the toxin can produce the characteristic rash of scarlet fever
- **erythromycin** protein synthesis inhibitor of the macrolide class that is often used as an alternative to penicillin
- **eschar** a localized mass of dead skin tissue
- **Etest** simple, rapid method for determining MIC, involving commercially available plastic strips that contain a gradient of an antimicrobial and are placed on an agar plate inoculated with a bacterial lawn
- **etiologic agent** the pathogen or substance responsible for causing a particular disease; causative agent
- **etiology** the science of the causes of disease

- **Eukarya** the domain of life that includes all unicellular and multicellular organisms with cells that contain membrane-bound nuclei and organelles
- **eukaryote** an organism made up of one or more cells that contain a membrane-bound nucleus and organelles
- **eukaryotic cell** has a nucleus surrounded by a complex nuclear membrane that contains multiple, rod-shaped chromosomes
- **eustachian tube** small passage between the nasopharynx and the middle ear that allows pressure to equalize across the tympanic membrane
- **eutrophs** microorganisms that require a copious amount of organic nutrients; also called copiotrophs
- **excystment** the process of emerging from a cyst
- **exergonic reaction** chemical reaction that does not require energy beyond activation energy to proceed; releases energy when the reaction occurs
- **exocytosis** the release of the contents of transport vesicles to the cell's exterior by fusion of the transport vesicle's membrane with the plasma membrane
- **exoenzyme** secreted enzyme that enhances the ability of microorganisms to invade host cells
- **exon** protein-coding sequence of a eukaryotic gene that is transcribed into RNA and spliced together to code for a polypeptide
- **exonuclease** enzymatic activity that removes RNA primers in DNA introduced by primase
- **exotoxin** biologically active product that causes adverse changes in the host cells
- **experimental epidemiology** the use of laboratory and clinical studies to directly study disease in a population
- **experimental study** a type of scientific study that involves manipulation of the study subjects by the researcher through application of specific treatments hypothesized to affect the outcome while maintaining rigorously controlled conditions
- **exposure** contact between potential pathogen and host; also called contamination or contact
- **extended-spectrum β -lactamases (ESBLs)** β -lactamases carried by some gram-negative bacteria that provide resistance to all penicillins,

cephalosporins, monobactams, and β -lactamase-inhibitor combinations, but not carbapenems

- **extensively drug resistant *Mycobacterium tuberculosis* (XDR-TB)** strains of *M. tuberculosis* that are resistant to rifampin and isoniazid, and also are resistant to any fluoroquinolone and at least one of three other drugs (amikacin, kanamycin, or capreomycin)
- **extracellular matrix** material composed of proteoglycans and fibrous proteins secreted by some eukaryotic cells that lack cell walls; helps multicellular structures withstand physical stresses and coordinates signaling from the external surface of the cell to the interior of the cell
- **extracellular polymeric substances (EPS)** hydrated gel secreted by bacteria in a biofilm containing polysaccharides, proteins, nucleic acids, and some lipids
- **extrachromosomal DNA** additional molecules of DNA distinct from the chromosomes that are also part of the cell's genome
- **extravasation** process by which leukocytes pass through capillary walls to reach infected tissue; also called diapedesis

F

- **F⁻ (recipient) cell** *E. coli* cell lacking the F plasmid and thus incapable of forming a conjugation pilus but capable of receiving the F plasmid during conjugation
- **F pilus (F pili)** specialized type of pilus that aids in DNA transfer between cells; conjugation pilus of *E. coli*
- **F plasmid (fertility factor)** bacterial plasmid in *E. coli* containing genes encoding the ability to conjugate, including genes encoding the formation of the conjugation pilus
- **F' plasmid** integrated F plasmid imprecisely excised from the chromosome; carries with it some chromosomal DNA adjacent to the integration site
- **F⁺ (donor) cell** *E. coli* cell containing the F plasmid, capable of forming a conjugation pilus
- **Fab region** arm of an antibody molecule that includes an antigen-binding site

- **facultative anaerobe** organism that grows better in the presence of oxygen but can proliferate in its absence
- **false negative** negative result to a test for an infection or condition (e.g., presence of antigen, antibody, or nucleic acid) when the infection or condition is actually present
- **false positive** positive result to a test for an infection or condition (e.g., presence of antigen, antibody, or nucleic acid) when the infection or condition is actually absent
- **fastidious organism** organism that has extensive growth requirements
- **fatty acid** lipid that contains long-chain hydrocarbons terminated with a carboxylic acid functional group
- **fatty acid methyl ester (FAME) analysis** technique in which the microbe's fatty acids are extracted, converted to volatile methyl esters, and analyzed by gas chromatography, yielding chromatograms that may be compared to reference data for identification purposes
- **Fc region** region on the trunk of an antibody molecule involved in complement activation and opsonization
- **feedback inhibition** mechanism of regulating metabolic pathway whereby the product of a metabolic pathway noncompetitively binds to an enzyme early on in the pathway, temporarily preventing the synthesis of the product
- **fermentation** process that uses an organic molecule as a final electron acceptor to regenerate NAD^+ from NADH such that glycolysis can continue
- **fever** system-wide sign of inflammation that raises the body temperature and stimulates the immune response
- **fifth disease** a highly contagious illness, more commonly affecting children, marked by a distinctive "slapped-cheek" rash and caused by parvovirus B19
- **fimbriae** filamentous appendages found by the hundreds on some bacterial cells; they aid adherence to host cells
- **fine focusing knob** a knob on a microscope that produces relatively small movements to adjust focus
- **fixation** the process by which cells are killed and attached to a slide
- **flagella** long, rigid, spiral structures used by prokaryotic cells for motility in aqueous environments; composed of a filament made of

flagellin, a hook, and motor (basal body) that are attached to the cell envelope

- **flagella staining** a staining protocol that uses a mordant to coat the flagella with stain until they are thick enough to be seen
- **flagellum (eukaryotic) (plural: flagella)** long, whip-like, filamentous external structure found on some eukaryotic cells; composed of microtubules in a 9+2 arrangement; used for locomotion
- **flavin adenine dinucleotide (FAD/FADH₂)** oxidized/reduced forms of an electron carrier in cells
- **flocculant** visible aggregation that forms between a substance in suspension (e.g., lipid in water) and antibodies against the substance
- **flow cytometry** technique analyzing cells for fluorescence intensity; specific subsets of cells are usually labeled in some way prior to the analysis
- **fluconazole** antifungal drug of the imidazole class that is administered orally or intravenously for the treatment of several types of systemic yeast infections
- **fluid mosaic model** refers to the ability of membrane components to move fluidly within the plane of the membrane, as well as the mosaic-like composition of the components
- **flukes** any of the parasitic nonsegmented flatworms (trematodes) that have an oral sucker and sometimes a second ventral sucker; they attach to the inner walls of intestines, lungs, large blood vessels, or the liver in human hosts
- **fluorescence microscope** a microscope that uses natural fluorochromes or fluorescent stains to increase contrast
- **fluorescence-activated cell sorter (FACS)** technique for using a flow cytometer to physically separate cells into two populations based on fluorescence intensity
- **fluorescent antibody (FA) techniques** suite of assays that use a fluorescently labeled antibody to bind to and so make an antigen easy to visualize
- **fluorescent enzyme immunoassay (FEIA)** EIA in which the substrate is a fluorogen that becomes fluorescent following reaction with the enzyme
- **fluorescent** the ability of certain materials to absorb energy and then immediately release that energy in the form of light

- **fluorochromes** chromophores that fluoresce (absorb and then emit light)
- **fluorogen** nonfluorescent molecule that becomes fluorescent on enzyme or laser activation
- **fluorophore** molecule that fluoresces when excited by light
- **fluoroquinolones** class of synthetic antimicrobials that inhibit the activity of DNA gyrase, preventing DNA replication
- **focal infection** infection in which the pathogen causes infection in one location that then spreads to a secondary location
- **focal length** the distance from the lens to the image point when the object is at a definite distance from the lens (this is also the distance to the focal point)
- **focal point** a property of the lens; the image point when light entering the lens is parallel (i.e., the object is an infinite distance from the lens)
- **foliose lichens** lichens that have lobes that may appear to resemble leaves
- **folliculitis** a skin infection characterized by localized inflammation of hair follicles, typically producing an itchy red rash
- **fomite** inanimate item that may harbor microbes and aid in disease transmission
- **foodborne disease** disease that is transmitted through contaminated food
- **fragmentation** newly formed cells split away from the parent filament in actinomycetes and cyanobacteria
- **frameshift mutation** mutation resulting from either an insertion or a deletion in a number of nucleotides that, if not a multiple of three, changes every amino acid after the mutation
- **free ribosome** eukaryotic 80S ribosome found in the cytoplasm; synthesizes water-soluble proteins
- **frequency** the rate of vibration for a light wave or other electromagnetic wave
- **fruticose lichens** lichens that are generally branched with a rounded appearance
- **functional groups** specific groups of atoms that may occur within a molecule, conferring specific chemical properties
- **fungi** (singular: fungus) any of various unicellular or multicellular eukaryotic organisms, typically having cell walls made out of chitin

- and lacking photosynthetic pigments, vascular tissues, and organs
- **fungicide** chemical or physical treatment that kills fungi
- **fungistatic** having the ability to inhibit fungal growth, generally by means of chemical or physical treatment
- **furuncle** a small, purulent skin lesion; sometimes called a boil
- **fusion inhibitor** antiviral drug that blocks the fusion of HIV receptors to the coreceptors required for virus entry into the cell, specifically, chemokine receptor type 5

G

- **Gammaproteobacteria** class of Proteobacteria that is very diverse and includes a number of human pathogens
- **gas gangrene** rapidly spreading infection of necrotic tissues caused by the gram-positive anaerobe *Clostridium perfringens* and other *Clostridium* spp.
- **gastritis** inflammation of the lining of the stomach
- **gastroenteritis** inflammation of the lining of the stomach and intestine
- **gene expression** production of proteins from the information contained in DNA through the processes of transcription and translation
- **gene gun** an apparatus that shoots gold or tungsten particles coated with recombinant DNA molecules at high speeds into plant protoplasts
- **gene silencing** a genetic engineering technique in which researchers prevent the expression of a particular gene by using small interfering RNAs (siRNAs) or microRNAs (miRNAs) to interfere with translation
- **gene therapy** a form of treatment for diseases that result from genetic mutations; involves the introduction of nonmutated, functional genes into the genome of the patient, often by way of a viral vector
- **generalized transduction** transfer of a random piece of bacterial chromosome DNA by the phage
- **generation time** see *doubling time*
- **genes** segments of DNA molecules that code for proteins or stable RNA molecules
- **genetic code** correspondence between mRNA nucleotide codons and the translated amino acids

- **genetic engineering** the direct alteration of an organism's genetics to achieve desirable traits
- **genital herpes** an STI caused by the herpes simplex virus
- **genital warts** soft, pink, irregular growths that develop in the external genitalia or anus as a result of human papillomavirus infection
- **genome** entire genetic content of a cell
- **genomic library** a repository of an organism's entire genome maintained as cloned fragments in the genomes of strains of a host organism
- **genomics** the study and comparison of entire genomes, including the complete set of genes, their nucleotide sequence and organization, and their interactions within a species and with other species
- **genotype** full collection of genes that a cell contains within its genome
- **germ theory of disease** the theory that many diseases are the result of microbial infection
- **germination** process of an endospore returning to the vegetative state
- **Ghon complex** calcified lesion containing *Mycobacterium tuberculosis*; forms in the lungs of patients with tuberculosis
- **giardiasis** intestinal infection caused by *Giardia lamblia*
- **gingivitis** inflammation of the gums that can cause bleeding
- **glial cell** assists in the organization of neurons, provides a scaffold for some aspects of neuron function, and aids in recovery from neural injury
- **glomerulonephritis** a type of kidney infection involving the glomeruli of the nephrons
- **glomerulus** capillary bed in the nephron of the kidney that filters blood to form urine
- **glycocalyx** cell envelope structure (either capsules or slime layer) outside the cell wall in some bacteria; allows bacteria to adhere to surfaces, aids in biofilm formation, and provides protection from predation
- **glycogen** highly branched storage polysaccharide in animal cells and bacteria
- **glycolipid** complex lipid that contains a carbohydrate moiety
- **glycolysis** first step in the breakdown of glucose, the most common example of which is the Embden-Meyerhoff-Parnas pathway,

producing two pyruvates, two NADH molecules, and two (net yield) ATP per starting glucose molecule

- **glycopeptides** class of antibacterials that inhibit cell wall synthesis by binding to peptidoglycan subunits and blocking their insertion into the cell wall backbone, as well as blocking transpeptidation
- **glycoprotein** conjugated protein with a carbohydrate attached
- **glycosidic bond** forms between the hydroxyl groups of two sugar molecules
- **Golgi apparatus** an organelle of the endomembrane system that is composed of a series of flattened membranous disks, called dictyosomes, each having a single lipid bilayer, that are stacked together; important in the processing of lipids and proteins
- **gonorrhea** a common STI of the reproductive system caused by *Neisseria gonorrhoeae*
- **graft-versus-host disease** specific type of transplantation reaction in which a transplanted immune system (e.g., a bone marrow transplant) contains APCs and T cells that are activated and attack the recipient's tissue
- **Gram stain procedure** a differential staining technique that distinguishes bacteria based upon their cell wall structure
- **granulocytes** leukocytes found in the peripheral blood that are characterized by numerous granules in the cytoplasm; granulocytes include neutrophils, eosinophils, and basophils
- **granuloma** walled-off area of chronically inflamed tissue containing microbial pathogens, macrophages, and cellular materials unable to be eliminated
- **granulomatous amoebic encephalitis (GAE)** serious brain infection of immunocompromised individuals caused by *Acanthamoeba* or *Balamuthia mandrillaris*
- **granzymes** proteases released from a natural killer cell that enter the cytoplasm of a target cell, inducing apoptosis
- **Graves disease** hyperthyroidism caused by an autoimmune disease affecting thyroid function
- **green nonsulfur bacteria** similar to green sulfur bacteria but use substrates other than sulfides for oxidation
- **green sulfur bacteria** phototrophic, anaerobic bacteria that use sulfide for oxidation and produce large amounts of green bacteriochlorophyll

- **growth curve** a graph modeling the number of cells in a culture over time
- **guanine** purine nitrogenous base found in nucleotides
- **Guillain-Barré syndrome** an autoimmune disease, often triggered by bacterial and viral infections, characterized by the destruction of myelin sheaths around neurons, resulting in flaccid paralysis
- **gummas** granulomatous lesions that develop in tertiary syphilis

H

- **hair follicle** a structure embedded in the dermis from which hair grows
- **halophile** organism that depends on high concentrations of salt in the environment to grow
- **halotolerant** organism that grows in the presence of high salt concentration but does not require it
- **Hansen's Disease** chronic bacterial infection of peripheral nervous tissues caused by the acid-fast bacterium, *Mycobacterium leprae*; also known as leprosy
- **hantavirus pulmonary syndrome** acute lung infection by a hantavirus following inhalation of aerosols from the urine or feces of infected rodents
- **haploid** having one copy of each chromosome
- **haptin** a molecule that is too small to be antigenic alone but becomes antigenic when conjugated to a larger protein molecule
- **hard chancre** a generally painless ulcer that develops at the site of infection in primary syphilis
- **Hashimoto thyroiditis** hypothyroidism caused by an autoimmune disease affecting thyroid function
- **healthcare-associated infection (HAI)** an infection acquired in a hospital or other health-care facility unrelated to the reason for which the patient was initially admitted; nosocomial infection
- **heavy chains** longest identical peptide chains in antibody molecules (two per antibody monomer), composed of variable and constant region segments
- **helical virus** cylindrical or rod shaped

- **helicase** enzyme that unwinds DNA by breaking the hydrogen bonds between the nitrogenous base pairs, using ATP
- **helminth** a multicellular parasitic worm
- **helper T cells** class of T cells that is the central orchestrator of the cellular and humoral defenses of adaptive immunity and the cellular defenses of innate immunity
- **hemagglutination** visible clumping of red blood cells that can be caused by some viruses, bacteria, and certain diseases in which antibodies are produced that bind to self-red blood cells
- **hematopoiesis** formation, development, and differentiation of blood cells from pluripotent hematopoietic stem cells
- **hematuria** condition in which there is blood in the urine
- **hemolysin** class of exotoxin that targets and lyses red blood cells, as well as other cells
- **hemolytic disease of the newborn (HDN)** type II hypersensitivity reaction that occurs when maternal anti-Rh antibodies cross the placenta and target fetal Rh⁺ red blood cells for lysis
- **hemolytic transfusion reaction (HTR)** condition resulting after an incompatible blood transfusion; caused by type II hypersensitivity reaction and destruction of red blood cells
- **hemorrhagic fever with renal syndrome** serious hemorrhagic fever caused by hantavirus infection
- **HEPA filter** high-efficiency particulate air filter with an effective pore size that captures bacterial cells, endospores, and viruses as air passes through, removing them from the air
- **hepatitis** inflammation of the liver
- **herd immunity** a reduction in disease prevalence brought about when few individuals in a population are susceptible to an infectious agent
- **herpes keratitis** eye infection caused by herpes simplex virus
- **herpes simplex virus type 2 (HSV-2)** the type of herpesvirus most commonly associated with genital herpes
- **herpetic gingivostomatitis** inflammation of the mouth and gums often caused by the HSV-1 virus
- **heterolactic fermentation** process producing a mixture of lactic acid, ethanol and/or acetic acid, and CO₂ as fermentation products; the microbes that do this use pentose phosphate pathway glycolysis, which is why they generate multiple fermentation products

- **heterotroph** organism that uses fixed organic carbon compounds as its carbon source
- **hexose monophosphate shunt** see *pentose phosphate pathway*
- **Hfr cell** *E. coli* cell in which an F plasmid has integrated into the host cell's chromosome
- **high G+C gram-positive bacteria** bacteria that have more than 50% guanine and cytosine nucleotides in their DNA
- **high-energy phosphate bond** bond between the negatively charged phosphate groups that holds a lot of potential energy
- **histamine** proinflammatory molecule released by basophils and mast cells in response to stimulation by other cytokines and chemical mediators
- **histones** DNA-binding proteins found in eukaryotes and archaea that aid in orderly packaging of chromosomal DNA
- **histoplasmosis** fungal disease caused by the dimorphic fungus *Histoplasma capsulatum*
- **holoenzyme** enzyme with a bound cofactor or coenzyme
- **holozoic** refers to protozoans that consume food particles through phagocytosis
- **homolactic fermentation** process producing only lactic acid as a fermentation product; the microbes that do this use Embden-Meyerhof-Parnas glycolysis
- **hookworm infection** soil-transmitted intestinal infection caused by the nematodes *Necator americanus* and *Ancylostoma doudenale*
- **horizontal direct transmission** movement of a pathogen from one host to another (excluding mother to embryo, fetus, or infant) in a population through physical contact or through droplet transmission
- **horizontal gene transfer** introduction of genetic material from one organism to another organism within the same generation
- **host range** the types of host cells that a particular virus is able to infect
- **HTST** high-temperature short-time pasteurization is a method of pasteurization commonly used for milk in which the milk is exposed to a temperature of 72 °C for 15 seconds
- **human African trypanosomiasis** serious infection caused by *Trypanosoma brucei* and spread by the bite of the tsetse fly

- **human granulocytic anaplasmosis** zoonotic tickborne disease caused by the obligate intracellular pathogen *Anaplasma phagocytophilum*
- **human immunodeficiency virus (HIV)** retrovirus responsible for acquired immune deficiency syndrome (AIDS) in humans
- **human papillomavirus (HPV)** a group of common sexually transmitted viruses that may be associated with genital warts or with cervical cancer
- **humanized monoclonal antibodies** chimeric antibodies with mouse variable regions and human constant regions
- **humoral immunity** adaptive immunity mediated by antibodies produced by B cells
- **hyaluronidase** enzyme produced by pathogens that degrades hyaluronic acid between adjacent cells in connective tissue
- **hybridization** the joining of two complementary single-stranded DNA molecules
- **hybridoma** clones of cell produced by fusing a normal B cell with a myeloma cell that is capable of producing monoclonal antibodies indefinitely
- **hydatid disease** cystic echinococcosis, an infection caused by the tapeworm *Echinococcus granulosus*
- **hydrophilic** “water loving”; refers to a polar molecule or portion of a molecule capable of strong attraction to water molecules
- **hydrophobic** “water fearing”; refers to a nonpolar molecule or portion of a molecule not capable of strong attraction to water molecules
- **hypersensitivity pneumonitis (HP)** type III and IV hypersensitivities in the lungs that are caused by environmental or occupational exposure to allergens such as mold and dust
- **hypersensitivity** potentially damaging immune response against an antigen
- **hyperthermophile** a microorganism that has an optimum growth temperature close to the temperature of boiling water
- **hypertonic medium** an environment in which the solute concentration outside a cell exceeds that inside the cell, causing water molecules to move out of the cell, resulting in crenation (shriveling) or plasmolysis.
- **hyphae** tubular, filamentous structures that makes up most fungi
- **hypodermis** the layer of tissue under the dermis, consisting primarily of fibrous and adipose connective tissue

- **hypotonic medium** an environment in which the solute concentration inside a cell exceeds that outside the cell, causing water molecules to move into the cell, possibly leading to swelling and possibly lysis

I

- **iatrogenic disease** disease caused by or acquired during a medical procedure
- **icosahedral** three-dimensional, 20-sided structure with 12 vertices
- **IgA** antibody dimer primarily found in breast milk, mucus, saliva, and tears
- **IgD** membrane-bound antibody monomer functioning as receptor on the surface of B cells
- **IgE** antibody monomer involved in defense against parasites and allergic reactions
- **IgG** antibody monomer most abundant in serum; able to cross placenta; most versatile class of antibody in terms of function
- **IgM** antibody that is a monomer when functioning as a receptor on surface of B cells but a pentamer when secreted in response to specific pathogens; first antibody to respond during primary and secondary responses
- **illuminator** the light source on a microscope
- **image point (focus)** a property of the lens and the distance of the object to the lens; the point at which an image is in focus (the image point is often called the focus)
- **imidazoles** class of antifungal drugs that inhibit ergosterol biosynthesis
- **immune complex** large group of antigens bound by antibodies; large enough to settle out of fluid suspension
- **immunochromatographic assay** assay in which fluids are pulled through test strips by capillary action and antigen captured by mobile antibody-colored bead conjugates; a second, fixed antibody localizes the colored bead, allowing visualization
- **immunocytochemistry (ICC)** staining technique in which cells are fixed and holes dissolved in the membrane to allow passage of labeled antibodies to bind specific intracellular targets

- **immuno-electrophoresis (IEP)** assay following protein electrophoresis (PAGE) of serum, in which antisera against specific serum proteins are added to troughs cut parallel to the electrophoresis track, causing the formation of precipitin arcs
- **immunofiltration** technique in which antibody or antigen can be concentrated by passing fluids through porous membranes, and target molecules are captured as they pass
- **immunofluorescence** a technique that uses a fluorescence microscope and antibody-specific fluorochromes to determine the presence of specific pathogens in a specimen
- **immunoglobulin** antibody
- **immunohistochemistry (IHC)** staining technique in which labeled antibodies are bound to specific cells in a tissue section
- **immunology** the study of the immune system
- **immunostain** use of EIA technology to deliver stain to particular cells in a tissue (immunohistochemistry) or specific targets within a cell (immunocytochemistry)
- **impetigo** a skin infection that may result in vesicles, blisters, or bullae especially around the mouth, commonly caused by *Staphylococcus aureus*, *S. pyogenes*, or a combination of both *S. aureus* and *S. pyogenes*
- **in vitro** outside the organism in a test tube or artificial environment
- **in vivo** inside the organism
- **inactivated vaccine** vaccine composed of whole pathogen cells or viruses that have been killed or inactivated through treatment with heat, radiation, or chemicals
- **incidence** the number of individuals with new infections of a particular disease in a given period of time
- **inclusion conjunctivitis** inflammation of the conjunctiva in newborns caused by *Chlamydia trachomatis* transmitted during childbirth
- **inclusions** prokaryotic cell cytoplasmic structures for storing specific nutrients and other resources needed by cells
- **incubation period** the first stage of acute disease, during which the pathogen begins multiplying in the host and signs and symptoms are not observable
- **indirect agglutination assay** assay that can be used to detect the agglutination of small latex beads; beads may be coated with antigen

when looking for the presence of specific antibodies, or with antibody when looking for the presence of antigen

- **indirect antiglobulin test (IAT)** see indirect Coombs' test
- **indirect contact transmission** transfer of an infectious agent between hosts through contact with a fomite
- **indirect Coombs' test** assay, performed *in vitro* prior to blood transfusions, that looks for antibodies against red blood cell antigens (other than the A and B antigens) that are unbound in a patient's serum
- **indirect ELISA** EIA in which an antigen from a pathogen is first attached to the wells of a microtiter plate; the antigen then captures antibodies from patient serum to determine whether the patient currently has or previously had the disease
- **indirect fluorescent antibody test** assay for antigen-specific antibodies wherein the antigen captures the antibody, which is subsequently detected using a labeled anti-immunoglobulin mAb
- **induced mutation** mutation caused by a mutagen
- **inducer** small molecule that either activates or represses transcription
- **inducible operon** bacterial operon, typically containing genes encoding enzymes in a degradative pathway, whose expression is induced by the substrate to be degraded when the substrate is available for the cell to use, but that is otherwise repressed in the absence of the substrate
- **induction** prophage DNA is excised from the bacterial genome
- **infection** the successful colonization of a microorganism within a host
- **infectious arthritis (septic arthritis)** inflammation of joint tissues in response to a microbial infection
- **infectious disease** disease caused by a pathogen
- **infectious mononucleosis** common and mild infection caused by Epstein-Barr virus (HHV-4) or cytomegalovirus (HHV-5); transmitted by direct contact with body fluids such as saliva
- **inflammation** innate nonspecific immune response characterized by erythema, edema, heat, pain, and altered function, typically at the site of injury or infection but sometimes becoming systemic.
- **influenza** highly contagious and acute viral disease of the respiratory tract caused by the influenza virus
- **initiation factors** proteins that participate in ribosome assembly during initiation

- **initiation of DNA replication** stage of replication during which various proteins bind to the origin of replication to begin the replication process
- **initiation of transcription** stage of transcription during which RNA polymerase binds to a promoter and transcription begins
- **initiation of translation** stage of translation during which an initiation complex composed of the small ribosomal subunit, the mRNA template, initiation factors, GTP, and a special initiator tRNA forms, and the large ribosomal subunit then binds to the initiation complex
- **inoculum** small number of cells added to medium to start a culture
- **inorganic phosphate (P_i)** single phosphate group in solution
- **insertion** type of mutation involving the addition of one or more bases into a DNA sequence
- **integrase inhibitors** antiviral drugs that block the activity of the HIV integrase responsible for recombination of a DNA copy of the viral genome into the host cell chromosome
- **intercalating agent** molecule that slides between the stacked nitrogenous bases of the DNA double helix, potentially resulting in a frameshift mutation
- **interference** distortion of a light wave due to interaction with another wave
- **interferons** cytokines released by cells that have been infected with a virus; stimulate antiviral responses in nearby cells as well as the cells secreting the interferons
- **interleukins** cytokines largely produced by immune system cells that help coordinate efforts against invading pathogens
- **intermediate filament** one of a diverse group of cytoskeletal fibers that act as cables within the cell and anchor the nucleus, comprise the nuclear lamina, or contribute to the formation of desmosomes
- **intermediate host** a host in which a parasite goes through some stages of its life cycle before migrating to the definitive host
- **intermittent common source spread** a mode of disease transmission in which every infection originates from the same source and that source produces infections for a period before stopping and then starting again
- **intertrigo** a rash that occurs in a skin fold

- **intestinal fluke** a trematode worm that infects the intestine, often caused by *Fasciolopsis buski*
- **intracellular targeting toxin** see *A-B exotoxin*
- **intrinsic growth rate** genetically determined generation time under specific conditions for a bacterial strain
- **intron** intervening sequence of a eukaryotic gene that does not code for protein and whose corresponding RNA sequences are removed from the primary transcript during splicing
- **intubation** placement of a tube into the trachea, generally to open the airway or to administer drugs or oxygen
- **in-use test** a technique for monitoring the correct use of disinfectants in a clinical setting; involves placing used, diluted disinfectant onto an agar plate to see if microbial colonies will grow
- **invasion** dissemination of a pathogen through local tissues or throughout the body
- **iodophor** compound in which iodine is complexed to an organic molecule, increasing the stability and efficacy of iodine as a disinfectant
- **ionizing radiation** high-energy form of radiation that is able to penetrate surfaces and sterilize materials by damaging microbial cell components and DNA
- **ischemia** condition marked by the inadequate flow of blood to the tissues
- **isograft** tissue grafted from one monozygotic twin to another
- **isohemagglutinins** IgM class antibodies produced against A or B red blood cell antigens
- **isomers** molecules that have the same atomic makeup but differ in the structural arrangement of the atoms
- **isoniazid** antimetabolite that inhibits biosynthesis of mycolic acid; used for the treatment of mycobacterial infections
- **isoprenoid** branched lipid derived from five-carbon isoprene molecules
- **isotonic medium** a solution in which the solute concentrations inside and outside the cell are approximately equal, thereby creating no net movement of water molecules across the cell membrane
- **ivermectin** antihelminthic drug of the avermectin class that binds to invertebrate glutamate-gated chloride channels to block neuronal

transmission in helminths

J

- **Japanese encephalitis** arboviral disease caused by the Japanese encephalitis virus (JEV) and endemic to Asia
- **jaundice** yellowish color of the skin and mucous membranes caused by excessive bilirubin caused by a failure of the liver to effectively process the breakdown of hemoglobin

K

- **keratin** a fibrous protein found in hair, nails, and skin
- **keratitis** inflammation of the cornea
- **keratoconjunctivitis** inflammation of both the cornea and the conjunctiva
- **kidney** organ that filters the blood, producing urine
- **Kinyoun technique** a method of acid-fast staining that does not use heat to infuse the primary stain, carbolfuchsin, into acid-fast cells
- **Kirby-Bauer disk diffusion test** simple, rapid method for determining susceptibility and resistance of a bacterial pathogen to antibacterial drugs. The test involves drug-impregnated disks placed on an agar plate inoculated with a bacterial lawn.
- **Koplik's spots** white spots that form on the inner lining of the cheek of patients with measles
- **Krebs cycle** cyclic pathway during which each two-carbon unit entering the cycle is further oxidized, producing three NADH, one FADH₂, and one ATP by substrate-level phosphorylation, releasing two CO₂ molecules and regenerating the molecule used in the first step; also called the *citric acid cycle* or the *tricarboxylic acid cycle*
- **kuru** rare form of transmissible spongiform encephalopathy endemic to Papua New Guinea

L

- **lacrimal duct** connects the lacrimal gland to the lacrimal sac
- **lacrimal gland** a gland situated above the eye that secretes tears
- **lacrimal punctum** opening in each upper and lower eyelid
- **lacrimal sac** a to a reservoir for tears; also known as the dacrocyst or tear sac
- **lag period** the time between antigen exposure and production of antibodies
- **lag phase** interval before exponential growth of a microbial population during which cells adjust to a new environment
- **lagging strand** strand of DNA made discontinuously by DNA polymerase
- **laryngitis** inflammation of the larynx
- **laryngopharynx** lower portion of the pharynx that connects to the larynx
- **larynx** region of the respiratory tract containing the vocal cords; also referred to as the voice box
- **latent disease** disease that goes into a dormant nonreplicative state after the acute disease and can persist in this state for years, with the risk of reactivation back into acute disease
- **latent virus** virus that remains dormant in the host genome
- **lateral flow test** see immunochromatographic assays
- **leading strand** strand of DNA made continuously in the 5' to 3' direction by DNA polymerase
- **Legionnaires disease** atypical pneumonia occurring in older individuals; caused by the inhalation of *Legionella pneumophila* aerosolized in water
- **leishmaniasis** protozoan infection caused by *Leishmania* spp. and transmitted by sand flies
- **leprosy** see *Hansen's disease*
- **leptospirosis** bacterial infection of the kidney caused by *Leptospira* spp.; may spread to the liver, lungs, brain, and other organs
- **leukocidin** class of exotoxin that targets and lyses leukocytes
- **leukocytes** white blood cells of various types, including granulocytes, lymphocytes, and monocytes

- **leukotrienes** lipid-based chemical mediators produced by leukocytes and other tissue cells; promote inflammation and allergic responses
- **lichen** symbiotic association of a fungus with an algae or cyanobacterium
- **ligation** repair of the sugar-phosphate backbone of the DNA, making the DNA molecule continuous
- **light chains** the shorter identical peptide chains of an antibody molecule (two per antibody monomer), composed of variable and constant region segments
- **light-dependent reaction** process by which energy from sunlight is absorbed by pigment molecules in photosynthetic membranes and converted into stored chemical energy in the forms of ATP and NADPH
- **light-harvesting complex** group of multiple proteins and associated pigments that each may absorb light energy to become excited, and transfer this energy from one pigment molecule to another until the energy is delivered to a reaction center pigment
- **light-independent reaction** process by which chemical energy, in the form of ATP and NADPH produced by the light-dependent reactions, is used to fix inorganic CO₂ into organic sugar; usually referred to as the Calvin-Benson cycle
- **lincomycin** naturally produced protein synthesis inhibitor of the lincosamide class that binds to the 50S subunit, inhibiting peptide bond formation
- **lincosamides** class of protein synthesis inhibitors that are similar to macrolides
- **linked recognition** a mechanism whereby a B cell and the helper T cell with which it interacts recognize the same antigen
- **lipase** extracellular enzyme that degrades triglycerides
- **lipid bilayer** biological membranes composed of two layers of phospholipid molecules with the nonpolar tails associating to form a hydrophobic barrier between the polar heads; also called unit membrane
- **lipid** macromolecule composed primarily of carbon and hydrogen; source of nutrients for organisms, a storage form for carbon and energy, a part of the structure of membranes, and may function as hormones, pharmaceuticals, fragrances, and pigments

- **lipopolysaccharide (LPS)** lipid molecules with attached sugars that are found as components of gram-negative outer membranes
- **lipoprotein** conjugated protein attached to a lipid
- **listeriosis** bacterial disease caused from the ingestion of the microbe *Listeria monocytogenes*
- **lithotroph** chemotroph that uses inorganic chemicals as its electron source; also known as chemoautotroph
- **live attenuated vaccine** vaccine with live pathogen that has been attenuated to become less virulent in order to produce an active but subclinical infection
- **liver fluke** a trematode worm that affects the bile duct of the liver, including *Fasciola hepatica* and *F. gigantica*
- **local infection** infection in one limited area
- **log phase** interval of growth when cells divide exponentially; also known as the exponential growth phase
- **loiasis** a disease caused by the parasitic *Loa loa* worm, which is transmitted by deerflies; adult worms live in the subcutaneous tissue and cause inflammation, swelling, and eye pain as they migrate through the skin and the conjunctiva of the eye
- **lophotrichous** having a single tuft of flagella located at one end of a bacterial cell
- **low G+C gram-positive bacteria** bacteria that have less than 50% of guanine and cytosine nucleotides in their DNA
- **lumen** space inside the cisternae of the endoplasmic reticulum in eukaryotic cells
- **Lyme disease** tickborne disease caused by the spirochete *Borrelia burgdorferi*
- **lymph nodes** bean-shaped organs situated throughout the body that contain areas called germinal centers, which are rich in B and T lymphocytes; also contain macrophages and dendritic cells for antigen presentation
- **lymphadenitis** inflammation of the lymph nodes
- **lymphangitis** inflammation of the lymphatic vessels
- **lymphogranuloma venereum** infection caused by *Chlamydia trachomatis* in tropical regions
- **lyophilization** rapid freezing, followed by placement under a vacuum, of a material so that water is lost by sublimation, thereby inhibiting

microbial growth

- **lysis** destruction of the host cell
- **lysogen** bacterium carrying the prophage
- **lysogenic conversion (phage conversion)** alteration of host characteristics or phenotypes due to the presence of phage
- **lysogenic cycle** life cycle of some phages in which the genome of the infecting phage is integrated into the bacterial chromosome and replicated during bacterial reproduction until it excises and enters a lytic phase of the life cycle
- **lysogeny** process of integrating the phage into the host genome
- **lysosome** an organelle of the endomembrane system that contains digestive enzymes that break down engulfed material such as foodstuffs, infectious particles, or damaged cellular components
- **lytic cycle** infection process that leads to the lysis of host cells

M

- **M protein** a streptococcal cell wall protein that protects the bacteria from being phagocytized. It is associated with virulence and stimulates a strong immune response
- **macrolides** class of protein synthesis inhibitors containing a large, complex ring structure that binds to the 50S subunit, inhibiting peptide bond formation
- **macromolecule** polymer assembled from individual units, monomers, that bind together like building blocks
- **macronucleus** larger nucleus in ciliate protists that have two nuclei; polyploid with a reduced genome of metabolic genes and derived from the micronucleus
- **macronutrient** element required in abundance in cells; account for approximately 99% of the cell's dry weight
- **macrophages** monocytes that have left the bloodstream and differentiated into tissue-specific phagocytes
- **mad cow disease** form of transmissible spongiform encephalopathy primarily affecting cattle; can be transmitted to humans by consumption of contaminated cattle products

- **magnetosomes** inclusions in certain bacterial cells containing magnetic iron oxide or iron sulfide, which allows bacteria to align along a magnetic field by magnetotaxis
- **magnetotaxis** directional movement of bacterial cells using flagella in response to a magnetic field
- **magnification** the power of a microscope (or lens) to produce an image that appears larger than the actual specimen, expressed as a factor of the actual size
- **major histocompatibility complex (MHC)** collection of genes that code for MHC glycoproteins expressed on the surface of all nucleated cells
- **malaise** a general feeling of being unwell
- **malaria** potentially fatal, mosquito-borne protozoan infection caused by several species of *Plasmodium* and characterized by a relapsing fever, nausea, vomiting, and fatigue
- **mast cells** granulocytes similar in origin and function to basophils, but residing in tissues
- **matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF)** technique in which the sample (e.g., a microbe colony) is mixed with a special matrix and irradiated with a high-energy laser to generate characteristic gaseous ions that are subjected to mass spectral analysis, yielding mass spectra that may be compared to reference data for identification purposes
- **maturation** assembly of viral components to produce a functional virus
- **mature naïve T cell** a T cell that has exited the thymus after thymic selection but has not yet been activated
- **maximum growth pH** highest pH value that an organism can tolerate for growth
- **maximum growth temperature** highest temperature at which a microorganism will divide or survive
- **maximum permissible oxygen concentration** highest concentration of oxygen at which an organism will grow
- **measles** highly contagious respiratory disease caused by the measles virus (MeV); marked by an intense macular rash and high fever; also known as rubeola

- **mebendazole** antihelminthic drug of the benzimidazole class that binds to helminthic β -tubulin, preventing microtubule formation
- **mechanical transmission** transfer of a pathogen between hosts by a mechanical vector
- **mechanical vector** an animal that transfers a pathogen from one host to another or from a reservoir to a host without being infected by the pathogen itself
- **median infectious dose (ID₅₀)** concentration of pathogen that will produce active infection in 50% of test animals inoculated
- **median lethal dose (LD₅₀)** concentration of pathogen that kills 50% of infected test animals
- **medulla** loosely packed layer of fungal filaments located underneath the cortex of a lichen
- **membrane attack complex (MAC)** ring structure formed from complement proteins C6 through C9 that penetrates the membranes of a targeted cell, causing cell lysis and death
- **membrane filtration** method to remove bacteria from liquid, typically heat-sensitive solutions, using filters with an effective pore size of 0.2 μm or smaller, depending on need
- **membrane filtration technique** known volumes are vacuum filtered aseptically through a membrane with a pore size small enough to trap microorganisms, which are counted after growth on plates
- **membrane-bound ribosome** 80S eukaryotic ribosome attached to rough endoplasmic reticulum
- **membrane-disrupting toxin** toxin that affects cell membrane function by either forming pores or disrupting the phospholipid bilayer
- **memory B cell** an activated and differentiated B cell that is programmed to respond to secondary exposures to a specific antigen
- **memory helper T cell** a long-lived T cell programmed to recognize and quickly mount a secondary response to a specific pathogen upon re-exposure
- **memory** the ability of the specific adaptive immune system to quickly respond to pathogens to which it has previously been exposed
- **meninges** membranes that surround the brain
- **meningitis** inflammation of the meningeal membranes that surround the brain

- **meningococcal meningitis** bacterial infection caused by *Neisseria meningitidis* that results in an inflammation of the meninges
- **meningoencephalitis** inflammatory response that involves both the brain and the membranes that surround it
- **MERS** Middle East respiratory syndrome; first described in Saudi Arabia in 2013; caused by a zoonotic coronavirus that results in flu-like symptoms
- **mesophile** a microorganism that grows best at moderate temperatures, typically between about 20 °C and 45 °C
- **metabolism** all of the chemical reactions inside of cells
- **metachromatic granule** a type of inclusion containing volutin, a polymerized inorganic phosphate that appears red when stained with methylene blue
- **metagenomics** the sequencing of genomic fragments from microbial communities, allowing researchers to study genes from a collection of multiple species
- **metatranscriptomics** the science of studying a collection of mRNA molecules produced from microbial communities; involves studying gene expression patterns from a collection of multiple species
- **methanogen** microorganism that produces gaseous methane
- **methicillin-resistant *Staphylococcus aureus* (MRSA)** pathogen resistant to all β -lactams through acquisition of a new low-affinity penicillin-binding protein, and often resistant to many other drug classes
- **metronidazole** antibacterial and antiprotozoan drug of the nitroimidazole class that is activated in anaerobic target cell and introduces DNA strand breakage, thus interfering with DNA replication in target cells
- **MHC I molecule** glycoprotein expressed on the surface of all nucleated cells and involved in the presentation of normal “self” antigens and foreign antigens from intracellular pathogens
- **MHC II molecule** glycoprotein expressed only on the surface of antigen-presenting cells and involved in the presentation of foreign antigens from pathogens ingested by phagocytosis
- **micelle** simple spherical arrangement of amphipathic lipid molecules with nonpolar tails aggregated within the interior and polar heads forming the outer surface

- **microaerophile** organism that requires oxygen at levels lower than atmospheric concentration
- **microarray analysis** a technique used to compare two samples of genomic DNA or cDNA; the DNA or cDNA fragments are immobilized on a chip and labeled with different fluorescent dyes, allowing for comparison of sequences or gene-expression patterns
- **microbe** generally, an organism that is too small to be seen without a microscope; also known as a microorganism
- **microbial death curve** graphical representation of the progress of a particular microbial control protocol
- **microbial ecology** study of the interactions between microbial populations microbiology the study of microorganisms
- **microbiome** all prokaryotic and eukaryotic microorganisms that are associated with a certain organism
- **microfilament** cytoskeletal fiber composed of actin filaments
- **microinjection** the direct injection of DNA into the cytoplasm of a eukaryotic cell using a glass micropipette
- **micronucleus** smaller nucleus in ciliate protists that have two nuclei; diploid, somatic, and used for sexual reproduction through conjugation
- **micronutrient** indispensable element present in cells in lower amounts than macronutrients; also called *trace element*
- **microorganism** generally, an organism that is too small to be seen without a microscope; also known as a microbe
- **microsporidia** fungi that lack mitochondria, centrioles, and peroxisomes; some can be human pathogens
- **microtiter plates** plastic dishes with multiple small wells
- **microtubule** hollow tube composed of tubulin dimers (α and β tubulin); the structural component of the cytoskeleton, centrioles, flagella, and cilia
- **miliary tuberculosis** hematogenous dissemination and spread of *Mycobacterium tuberculosis* from tubercles
- **minimal bactericidal concentration (MBC)** lowest antibacterial drug concentration that kills $\geq 99.9\%$ of a starting inoculum of bacteria
- **minimal inhibitory concentration (MIC)** lowest concentration of an antibacterial drug that inhibits visible growth of a bacterial strain
- **minimum growth pH** lowest pH value that an organism can tolerate for growth

- **minimum growth temperature** lowest temperature at which a microorganism will divide or survive
- **minimum permissible oxygen concentration** lowest concentration of oxygen at which an organism will grow
- **missense mutation** point mutation that results in a different amino acid being incorporated into the resulting polypeptide
- **mitochondrial matrix** the innermost space of the mitochondrion enclosed by two membranes; the location of many metabolic enzymes as well as the mitochondrial DNA and 70S ribosomes
- **mitochondrion (plural: mitochondria)** large, complex organelle that is the site of cellular respiration in eukaryotic cells
- **mode of action** way in which a drug affects a microbe at the cellular level
- **moist-heat sterilization** protocol that involves steam under pressure in an autoclave, allowing the steam to reach temperatures higher than the boiling point of water
- **mold** a multicellular fungus, typically made up of long filaments
- **molecular cloning** the purposeful fragmentation of DNA followed by attachment to another piece of DNA to produce a recombinant molecule, followed by introduction of this recombinant molecule into an easily manipulated host to allow for the creation of multiple copies of a gene of interest
- **monoclonal antibodies (mAbs)** antibodies produced *in vitro* that only bind to a single epitope
- **monocular** having a single eyepiece
- **monocytes** large, agranular, mononuclear leukocytes found in the peripheral blood; responsible for phagocytosis of pathogens and damaged cells
- **monoecious** refers to sexually reproducing organisms in which individuals have both male and female reproductive organs
- **monomer** small organic molecule that binds with like molecules, forming a polymer or macromolecule
- **monosaccharide** monomer for the synthesis of carbohydrate polymers; the simplest carbohydrate, called a *simple sugar*
- **monotrichous** having one flagellum, typically located on one end of the bacterial cell
- **morbidity** a state of illness

- **Morbidity and Mortality Weekly Report (MMWR)** the trade/industry publication for epidemiologists, reporting US public health data compiled by the CDC
- **morbidity rate** the number of cases of a disease expressed as a percentage of the population or number per standard part of the population, such as 100,000
- **mordant** a chemical added to a specimen that sets a stain
- **mortality** death
- **mortality rate** the number of deaths from a disease expressed as a percentage of the population or number per standard part of the population, such as 100,000
- **most probable number (MPN)** statistical value representing the viable bacterial population in a sample obtained after a series of dilutions and multiple tube inoculations
- **mRNA** short-lived type of RNA that serves as the intermediary between DNA and the synthesis of protein products
- **mucociliary escalator** system by which mucus and debris are propelled up and out of the respiratory tract by the beating of respiratory cilia and the mechanical actions of coughing or swallowing
- **mucormycosis** rare form of pneumonia that can be caused by an invasive infection of different fungi in the order Mucorales, such as *Rhizopus* or *Mucor*
- **mucous membrane** moist layer of epithelial cells and interspersed goblet cells that lines the inner surfaces of the body, usually bathed in antimicrobial secretions from the cells of the membrane
- **mucus** viscous secretion produced by cells and glands in various mucous membranes throughout the body; helps trap and remove microbes and debris from the body
- **multidrug-resistant microbes (MDR)** group of pathogens that carry one or more resistance mechanisms, making them resistant to multiple antimicrobials; also called superbugs
- **multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB)** strains of *M. tuberculosis* that are resistant to both rifampin and isoniazid, the drug combination typically prescribed for the treatment of tuberculosis
- **multiple sclerosis** autoimmune attack on the myelin sheaths and nerve cells in the central nervous system

- **mumps** a viral illness that causes swelling of the parotid glands; rare in the United States because of effective vaccination
- **murine typhus** fleaborne infection caused by *Rickettsia typhi* and characterized by fever, rash, and pneumonia
- **mutagen** type of chemical agent or radiation that can induce mutations
- **mutant** organism harboring a mutation that often has a recognizable change in phenotype compared to the wild type
- **mutation** heritable change in the DNA sequence of an organism
- **mutualism** type of symbiosis in which two populations benefit from, and depend on, each other
- **myasthenia gravis** autoimmune disease affecting the acetylcholine receptors in the neuromuscular junction, resulting in weakened muscle contraction capability
- **mycelium** vegetative network of branched, tubular hyphae
- **mycolic acids** waxy molecules associated with peptidoglycan in some gram-positive, acid-fast bacteria, chiefly mycobacteria
- **mycology** the study of fungi
- **Mycoplasma pneumonia** also known as walking pneumonia; a milder form of atypical pneumonia caused by *Mycoplasma pneumoniae*
- **mycoses (mycosis, sing.)** refers to diseases caused by fungi
- **mycotoxin** biologically active product of pathogenic fungi that causes adverse changes in the host cells
- **myelin sheath** insulating layer that surrounds the axon of some neurons and helps to promote signal propagation
- **myocarditis** inflammation of the heart muscle tissues

N

- **naïve mature B cell** a B cell that has not yet been activated
- **naked virus** virus composed of a nucleic acid core, either DNA or RNA, surrounded by a capsid
- **nalidixic acid** member of the quinolone family that functions by inhibiting the activity of DNA gyrase, blocking DNA replication
- **narrow-spectrum antimicrobial** drug that targets only a specific subset of microbes
- **nasal cavity** air-filled space in the skull immediately behind the nose

- **nasolacrimal duct** tear duct connecting the lacrimal glands to the nasal cavity
- **nasopharynx** part of the upper throat (pharynx) extending from the posterior nasal cavity; carries air inhaled through the nose
- **native structure** three-dimensional structure of folded fully functional proteins
- **natural active immunity** immunity that develops as a result of natural infection with a pathogen
- **natural antibiotic** antimicrobial compound that is produced naturally by microorganisms in nature
- **natural killer cells (NK cells)** lymphoid cells that recognize and destroy abnormal target cells by inducing apoptosis
- **natural passive immunity** transfer of maternal antibodies from mother to fetus (transplacentally) or infant (via breastmilk)
- **necrotizing fasciitis** a serious infection, also known as flesh-eating disease, that leads to rapid destruction of tissue through the action of exotoxin A; it can be caused by *S. pyogenes* or several other bacterial species
- **negative (–) single-strand RNA (–ssRNA)** a viral RNA strand that cannot be translated until it is replicated into positive single-strand RNA by viral RNA-dependent RNA polymerase
- **negative stain** a stain that produces color around the structure of interest while not coloring the structure itself
- **Nematoda** phylum comprising roundworms
- **neonatal herpes** herpes infection of the newborn, generally caused by infection during birth
- **neonatal meningitis** meningitis caused by Group B streptococcus and occurring primarily in neonates (less than 2 months old)
- **neonatal tetanus** tetanus acquired through infection of the cut umbilical cord
- **neurocysticercosis** parasitic invasion of brain tissues by the larvae of the pork tapeworm, *Taenia solium*
- **neuromycosis** any fungal infection of the nervous system
- **neuron** specialized cell found throughout the nervous system that transmits signals through the nervous system using electrochemical processes

- **neuropathy** numbness or tingling sensation caused by damage to peripheral nerves
- **neurotoxoplasmosis** disease caused by the invasion of brain tissues by the protozoan *Toxoplasma gondii*; typically only affects immunocompromised patients
- **neurotransmitter** compound that is released at the synapse of neurons to stimulate or suppress the actions of other cells
- **neutralism** type of symbiosis that does not affect either of the two populations
- **neutralization** binding of an antibody to a pathogen or toxin, preventing attachment to target cells
- **neutrophile** organism that grows best at a near a neutral pH of 6.5–7.5
- **neutrophils** leukocytes with a multilobed nucleus found in large numbers in peripheral blood; able to leave the bloodstream to phagocytose pathogens in infected tissues; also called polymorphonuclear neutrophils (PMNs)
- **next generation sequencing** a group of automated techniques used for rapid DNA sequencing
- **nicotine adenine dinucleotide (NAD⁺/NADH)** oxidized/reduced forms of an electron carrier in cells
- **nicotine adenine dinucleotide phosphate (NADP⁺/NADPH)** oxidized/reduced forms of an electron carrier in cells
- **nitrogen fixation** bacterial biochemical pathways that incorporate inorganic nitrogen gas into organic forms more easily used by other organisms
- **nitrogenous base** nitrogen-containing ring structure within a nucleotide that is responsible for complementary base pairing between nucleic acid strands
- **noncoding DNA** regions of an organism's genome that, unlike genes, do not encode proteins
- **noncommunicable disease** disease that is not transmitted from one person to another
- **noncompetitive (allosteric) inhibitor** molecule that binds to allosteric sites, inducing a conformational change in the enzyme's structure that prevents it from functioning
- **noncritical item** object that may contact intact skin but does not penetrate it; requires cleanliness but not a high level of disinfection

- **noncyclic photophosphorylation** pathway used in photosynthetic organisms when both ATP and NADPH are required by the cell
- **nonenveloped virus** naked virus
- **nongonococcal urethritis (NGU)** a nonspecific infection of the urethra that is not caused by *Neisseria gonorrhoeae*
- **noninfectious disease** disease caused by something other than an infectious agent (e.g., genetics, environment, nutritional deficiencies)
- **nonionizing radiation** low-energy radiation, like ultraviolet light, that can induce dimer formation between two adjacent pyrimidine bases, resulting in DNA polymerase stalling and possible formation of a frameshift mutation
- **nonsense mutation** point mutation that converts a codon encoding an amino acid (a sense codon) into a stop codon (a nonsense codon)
- **nontreponemal serologic tests** qualitative and quantitative indirect diagnostic tests for syphilis
- **northern blot** a technique in molecular genetics used to detect the amount of RNA made by gene expression within a tissue or organism sample; RNA fragments within a sample are separated by agarose gel electrophoresis, immobilized on a membrane, and then exposed to a specific DNA probe labeled with a radioactive or fluorescent molecular beacon to aid in detection
- **nosocomial disease** disease acquired in a hospital setting
- **notifiable disease** a disease for which all cases must legally be reported to regional, state, and/or federal public health agencies
- **nuclear envelope** (also called the nuclear membrane) a structure defining the boundary of the nucleus; composed of two distinct lipid bilayers that are contiguous with each other and with the endoplasmic reticulum
- **nuclear lamina** a meshwork of intermediate filaments (mainly lamins) found just inside the nuclear envelope; provides structural support to the nucleus
- **nucleic acid** class of macromolecules composed of nucleotide monomers polymerized into strands
- **nucleoid** concentrated area of DNA genome and associated proteins found in a prokaryotic cell that is not surrounded by a membrane
- **nucleoid-associated protein (NAP)** protein that assists in the organization and packaging of the chromosome in prokaryotic cells

- **nucleolus** a dense region within the nucleus where ribosomal RNA biosynthesis occurs and preribosomal complexes are made
- **nucleoside analog** chemical that is structurally similar to a normal nucleotide base that can be incorporated into DNA instead of normal bases during replication but that has different base pairing rules than the normal base for which it was substituted, inducing mutation
- **nucleotide excision repair (dark repair)** enzymatic mechanism to repair pyrimidine dimers by cutting the dimer-containing DNA strand on both sides of dimer, removing the intervening strand and replacing the bases with the correct ones
- **nucleotide** nucleic acid monomer composed of a pentose sugar, a phosphate group, and a nitrogenous base
- **nucleus** a membrane-bound structure of eukaryotic cells that houses the DNA genome
- **numerical aperture** a measure of a lens's ability to gather light

O

- **objective lenses** on a light microscope, the lenses closest to the specimen, typically located at the ends of turrets
- **obligate aerobe** organism that requires oxygen for growth
- **obligate anaerobe** organism that dies in the presence of oxygen
- **obligate intracellular pathogen** microorganism that cannot synthesize its own ATP and, therefore, must rely on a host cell for energy; behaves like a parasite when inside a host cell, but is metabolically inactive outside of a host cell
- **observational study** a type of scientific study that involves measurement of study subjects on variables hypothesized to be associated with the outcome of interest, but without any manipulation of the subjects
- **ocular lens** on a microscope, the lens closest to the eye (also called an eyepiece)
- **oil immersion lens** a special objective lens on a microscope designed to be used with immersion oil to improve resolution
- **Okazaki fragment** short fragment of DNA made during lagging strand synthesis

- **oligopeptide** peptide having up to approximately 20 amino acids
- **oligotroph** organism capable of living in low-nutrient environments
- **opacity** the property of absorbing or blocking light
- **operator** DNA sequence located between the promoter region and the first coding gene to which a repressor protein can bind
- **operon** a group of genes with related functions often found clustered together within the prokaryotic chromosome and transcribed under the control of a single promoter and operator repression sequence
- **ophthalmia neonatorum** inflammation of the conjunctiva in newborns caused by *Neisseria gonorrhoeae* transmitted during childbirth
- **opisthotonos** characteristic symptom of tetanus that results in uncontrolled muscular spasms and backward arching of the neck and spine
- **opportunistic pathogen** microorganism that can cause disease in individuals with compromised host defenses
- **opsonin** any molecule that binds to and coats the outside of a pathogen, identifying it for destruction by phagocytes (examples include antibodies and the complement proteins C3b and C4b)
- **opsonization** process of coating a pathogen with a chemical substance (an opsonin) that allows phagocytic cells to recognize, engulf, and destroy the pathogen more easily
- **optimum growth pH** the pH at which an organism grows best
- **optimum growth temperature** the temperature at which a microorganism's growth rate is highest
- **optimum oxygen concentration** the ideal concentration of oxygen for a particular microorganism
- **oral herpes** an infection caused by herpes simplex virus that results in cold sores, most commonly on and around the lips
- **oral thrush** *Candida* infection of the mouth
- **orchitis** inflammation of one or both of the testes
- **organic molecule** composed primarily of carbon; typically contains at least one carbon atom bound to one or more hydrogen atoms
- **organotroph** chemotroph that uses organic molecules as its electron source; also known as chemoheterotroph
- **origin of replication** specific nucleotide sequence where replication begins

- **oropharynx** area where air entering mouth enters the pharynx
- **osmosis** diffusion of water across a semipermeable membrane
- **osmotic pressure** the force or pressure generated by water diffusing across a semipermeable membrane, driven by differences in solute concentration across the membrane
- **osteomyelitis** inflammation of bone tissue
- **otitis externa** an infection of the external ear canal, most commonly caused by *Pseudomonas aeruginosa*; often called swimmer's ear
- **otitis** inflammation of the ear
- **otitis media with effusion** accumulation of fluid inside the middle ear with or without infection
- **Ouchterlony assay** test in which antigen and antisera are added to neighboring wells in an agar gel, allowing visualization of precipitin arcs
- **outer membrane** a phospholipid bilayer external to the peptidoglycan layer found in gram-negative cell walls
- **oxazolidinones** class of synthetic protein synthesis inhibitors that interfere with formation of the initiation complex for translation and prevent translocation of the growing protein from the ribosomal A site to the P site
- **oxidation reaction** chemical reaction that removes electrons (often as part of H atoms) from donor molecules, leaving them oxidized
- **oxidative phosphorylation** mechanism for making ATP that uses the potential energy stored within an electrochemical gradient to add P_i to ADP
- **oxygenic photosynthesis** type of photosynthesis found in plants, algae, and cyanobacteria, and in which H_2O is used as the electron donor to replace an electron lost by a reaction center pigment, resulting in oxygen as a byproduct

P

- **P (peptidyl) site** functional site of an intact ribosome that binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA

- **palatine tonsil** lymphoid tissue located near the oropharynx
- **pandemic disease** an epidemic that is worldwide as opposed to regional
- **papilloma** growth on the skin associated with infection by any of the human papilloma viruses (HPV); commonly known as a wart
- **paracrine function** refers to a cytokine signal released from a cell to a receptor on a nearby cell
- **parasitism** type of symbiosis in which one population benefits while harming the other parasitology the study of parasites
- **parenteral route** means of entry by a pathogen through skin or mucous membranes when these barriers are breached
- **paroxysmal stage** most serious stage of pertussis (whooping cough), characterized by severe and prolonged coughing spells
- **passive carrier** an individual capable of transmitting a pathogen to another individual without becoming infected
- **passive immunity** adaptive immune defenses received from another individual or animal
- **pasteurization** form of microbial control using heat that is applied to foods; kills pathogens and reduces the number of spoilage-causing microbes while maintaining food quality
- **pathogen** a disease-causing microorganism
- **pathogen-associated molecular patterns (PAMPs)** common molecular motifs found on pathogens
- **pathogenicity** ability of a microbial agent to cause disease
- **pattern recognition receptors (PRRs)** receptors on the surface or in the interior of phagocytic cells that bind to pathogen-associated molecular patterns (PAMPs)
- **pellicle** structure that underlies the plasma membrane in protists, providing additional support
- **pelvic inflammatory disease (PID)** infection of the female reproductive organs that may spread from the vagina to the cervix, uterus, fallopian tubes, and ovaries
- **penetration** entry of phage or virus into a host cell through injection, endocytosis, or membrane fusion
- **penicillin** β -lactam antibacterial that was the first cell wall synthesis inhibitor developed

- **penis** external genital organ in males through which urine and semen are discharged
- **pentamidine** antiprotozoan drug that appears to degrade kDNA in target cells, as well as inhibit protein synthesis
- **pentose phosphate pathway (PPP)** alternative glycolytic pathway that produces intermediates used for the biosynthesis of nucleotides and amino acids; also called the *phosphogluconate pathway* or the *hexose monophosphate shunt*
- **peptic ulcer** an ulcer in the lining of the stomach or duodenum, often associated with *Helicobacter pylori*
- **peptide bond** bond between the carboxyl group of one amino acid and the amine group of another; formed with the loss of a water molecule
- **peptidoglycan (murein)** the polymer of alternating N-acetylmuramic acid NAM and N-acetylglucosamine (NAG) subunits linked together by peptide chains; a major constituent of bacterial cell walls
- **peptidyl transferase** RNA-based ribozyme that is part of the 50S ribosomal subunit and catalyzes formation of the peptide bond between the amino acid bound to a tRNA and the growing polypeptide chain
- **perforin** compound released from a natural killer cell that creates pores in the target cell through which other toxins (particularly granzymes) can gain access to the cytoplasm
- **pericarditis** inflammation of the sac that surrounds the heart
- **period of convalescence** fifth stage of acute disease, during which the patient returns to normal function
- **period of decline** fourth stage of disease, during which the number of pathogens present in the host decreases, along with signs and symptoms of disease
- **period of illness** third stage of acute disease, during which the number of pathogens present in the host is greatest and the signs and symptoms of disease are most severe
- **periodontal disease** a condition in which the gums are inflamed and may erode
- **periodontitis** inflammation of the gums that is more severe than gingivitis, spreading deeper into the tissues
- **peripheral nervous system** network of neurons that connects the CNS with organs, sensory organs, and muscles throughout the body

- **peripheral tolerance** mechanism by which regulatory T cells inhibit self-reactive immune responses in T cells that have already exited the thymus
- **periplasmic space** the space between the cell wall and the plasma membrane, primarily in gram-negative bacteria
- **peristalsis** muscular contractions of the gastrointestinal tract that propel ingested material through the stomach, intestines, and, eventually, through the rectum and out of the body
- **peritrichous** having numerous flagella covering the entire surface of a bacterial cell
- **peroxidase** enzyme that catalyzes the detoxification of peroxides
- **peroxisome** in eukaryotic cells, a membrane-bound organelle (not part of the endomembrane system) that produces hydrogen peroxide to break down various types of molecules; also plays a role in lipid biosynthesis
- **peroxygen** type of strong oxidizing agent that causes free radical formation in cells; can be used as a disinfectant or antiseptic
- **persister** dormant cell that survives in the death phase and is resistant to most antibiotics
- **pertussis** contagious illness caused by *Bordetella pertussis* that causes severe coughing fits followed by a whooping sound during inhalation; commonly known as whooping cough
- **pertussis toxin** main virulence factor accounting for the symptoms of whooping cough
- **petechiae** small red or purple spots on the skin that result from blood leaking out of damaged vessels
- **Petroff-Hausser counting chamber** calibrated slide that allows counting of bacteria in a specific volume under a microscope
- **Peyer's patches** lymphoid tissue in the ileum that monitors and fights infections
- **phagemid** a plasmid capable of being replicated as a plasmid and also incorporated into a phage head
- **phagocytosis** a type of endocytosis in which large particles are engulfed by membrane invagination, after which the particles are enclosed in a pocket, which is pinched off from the membrane to form a vacuole

- **phagolysosome** compartment in a phagocytic cell that results when the phagosome is fused with the lysosome, leading to the destruction of the pathogens inside
- **phagosome** compartment in the cytoplasm of a phagocytic cell that contains the phagocytosed pathogen enclosed by part of the cell membrane
- **pharmacogenomics (toxicogenomics)** the evaluation of the effectiveness and safety of drugs on the basis of information from an individual's genomic sequence as well as examination of changes in gene expression in response to the drug
- **pharyngitis** inflammation of the pharynx
- **pharynx** region connecting the nose and mouth to the larynx: the throat
- **phase-contrast microscope** a light microscope that uses an annular stop and annular plate to increase contrast
- **phenol coefficient** measure of the effectiveness of a chemical agent through comparison with that of phenol on *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi
- **phenolics** class of chemical disinfectants and antiseptics characterized by a phenol group that denatures proteins and disrupts membranes
- **phenotype** observable characteristics of a cell or organism
- **phosphodiester bonds** linkage whereby the phosphate group attached to the 5' carbon of the sugar of one nucleotide bonds to the hydroxyl group of the 3' carbon of the sugar of the next nucleotide
- **phosphogluconate pathway** see *pentose phosphate pathway*
- **phospholipase** enzyme that degrades phospholipids
- **phospholipid** complex lipid that contains a phosphate group
- **phospholipid-derived fatty acids (PLFA) analysis** technique in which membrane phospholipids are saponified to release the fatty acids of the phospholipids, which can be subjected to FAME analysis for identification purposes
- **phosphorescence** the ability of certain materials to absorb energy and then release that energy as light after a delay
- **photosynthesis** process whereby phototrophic organisms convert solar energy into chemical energy that can then be used to build carbohydrates

- **photosynthetic pigment** pigment molecule used by a cell to absorb solar energy; each one appears the color of light that it transmits or reflects
- **photosystem** organized unit of pigments found within a photosynthetic membrane, containing both a light-harvesting complex and a reaction center
- **phototaxis** directional movement using flagella in response to light
- **phototroph** organism that gets its energy from light
- **phototrophic bacteria** nontaxonomic group of bacteria that use sunlight as their primary source of energy
- **phylogeny** the evolutionary history of a group of organisms
- **phytoplankton** photosynthetic plankton
- **pia mater** fragile and innermost membrane layer surrounding the brain
- **pili** long protein extensions on the surface of some bacterial cells; specialized F or sex pilus aids in DNA transfer between cells
- **pinocytosis** a type of endocytosis in which small dissolved materials are endocytosed into smaller vesicles
- **plague** infectious epidemic disease caused by *Yersinia pestis*
- **plankton** microscopic organisms that float in the water and are carried by currents; they may be autotrophic (phytoplankton) or heterotrophic (zooplankton)
- **planktonic** free-floating or drifting in suspension
- **plantibodies** monoclonal antibodies produced in plants that are genetically engineered to express mouse or human antibodies
- **plaque** clear area on bacterial lawn caused by viral lysis of host cells
- **plasma cell** activated and differentiated B cell that produces and secretes antibodies
- **plasma** fluid portion of the blood that contains all clotting factors
- **plasma membrane** (also called the cell membrane or cytoplasmic membrane) lipid bilayer with embedded proteins that defines the boundary of the cell
- **plasmalemma** protist plasma membrane
- **plasmid** small, circular, double-stranded DNA molecule that is typically independent from the bacterial chromosome
- **plasmolysis** the separation of the plasma membrane away from the cell wall when a cell is exposed to a hypertonic environment

- **platelets** cell fragments in the peripheral blood that originate from megakaryocyte cells in the bone marrow; also called thrombocytes
- **Platyhelminthes** phylum comprising flatworms
- **pleconaril** an antiviral drug targeting picornaviruses that prevents the uncoating of virus particles upon their infection of host cells
- **pleomorphic** able to change shape
- **pneumococcal meningitis** bacterial infection caused by *Streptococcus pneumoniae* that results in an inflammation of the meninges
- ***Pneumocystis pneumonia*** common pulmonary infection in patients with AIDS; caused by *P. jirovecii*
- **pneumonia** pulmonary inflammation that causes the lungs to fill with fluids
- **pneumonic plague** rare form of plague that causes massive hemorrhages in the lungs and is communicable through aerosols
- **point mutation** mutation, most commonly a base substitution, that affects a single base pair
- **point source spread** a form of common source spread in which the transmission of a disease from the source occurs for a brief period that is less than the pathogen's incubation period
- **polar tubule** a tube-like structure produced by spores of parasitic Microsporidia fungi that pierces host cell membranes
- **poliomyelitis (polio)** disease caused by an infection of the enteric polio virus characterized by inflammation of the motor neurons of the brain stem and spinal cord; can result in paralysis
- **poly-A tail** string of approximately 200 adenine nucleotides added to the 3' end of a eukaryotic primary mRNA transcript to stabilize it
- **polyacrylamide gel electrophoresis (PAGE)** a method for separating populations of proteins and DNA fragments during Sanger sequencing of varying sizes by differential migration rates caused by a voltage gradient through a vertical gel matrix
- **polycistronic mRNA** single mRNA molecule commonly produced during prokaryotic transcription that carries information encoding multiple polypeptides
- **polyclonal antibodies** antibodies produced in a normal immune response, in which multiple clones of B cells respond to many different epitopes on an antigen

- **polyenes** class of antifungal drugs that bind to ergosterol to form membrane pores, disrupting fungal cell membrane integrity
- **polyhedral virus** virus with a three-dimensional shape with many facets
- **polyhydroxybutyrate (PHB)** a type of cellular inclusion surrounded by a phospholipid monolayer embedded with protein
- **polylinker site** or **multiple cloning site (MCS)** a short sequence containing multiple unique restriction enzyme recognition sites that are used for inserting foreign DNA into the plasmid after restriction digestion of both the foreign DNA and the plasmid
- **polymer** macromolecule composed of individual units, monomers, that bind together like building blocks.
- **polymerase chain reaction (PCR)** an *in vitro* molecular technique that rapidly amplifies the number of copies of specific DNA sequences to make the amplified DNA available for other analyses
- **polymorphonuclear neutrophil (PMN)** see *neutrophils*
- **polymyxins** lipophilic polypeptide antibiotics that target the lipopolysaccharide component of gram-negative bacteria and ultimately disrupt the integrity of their outer and inner membranes
- **polypeptide** polymer having from approximately 20 to 50 amino acids
- **polyphyletic** refers to a grouping of organisms that is not descended from a single common ancestor
- **polyribosome (polysome)** structure including an mRNA molecule that is being translated by multiple ribosomes concurrently
- **polysaccharide** polymer composed of hundreds of monosaccharides linked together by glycosidic bonds; also called *glycans*
- **portal of entry** anatomical feature of the body through which pathogens can enter host tissue
- **portal of exit** anatomical feature of the body through which pathogens can leave diseased individual
- **positive (+) strand** viral RNA strand that acts like messenger RNA and can be directly translated inside the host cell
- **positive stain** a stain that colors the structure of interest
- **pour plate method** a technique used for inoculating plates with diluted bacterial samples for the purpose of cell counting; cells are mixed with warm liquid agar before being poured into Petri dishes

- **praziquantel** antihelminthic drug that induces a calcium influx into tapeworms, leading to spasm and paralysis
- **precipitin** complex lattice of antibody and antigen that becomes too large to stay in solution
- **precipitin ring test** assay in which layers of antisera and antigen in a test tube form precipitin at the interface of the two solutions
- **prevalence** the total number or proportion of individuals in a population ill with a specific disease
- **primary amoebic meningoencephalitis (PAM)** acute and deadly parasitic infection of brain tissues by the amoeba *Naegleria fowleri*
- **primary antibody** in a sandwich ELISA, the antibody that is attached to wells of a microtiter plate to capture antigen from a solution, or in an indirect ELISA, the antigen-specific antibody present in a patient's serum
- **primary cell culture** cells taken directly from an animal or plant and cultured in vitro
- **primary immunodeficiency** genetic condition that results in impaired immune function
- **primary infection** initial infection produced by a pathogen
- **primary lymphoid tissue** one of two types of lymphatic tissue; comprises bone marrow and the thymus
- **primary pathogen** microorganism that can cause disease in the host regardless of the effectiveness of the host's immune system
- **primary response** the adaptive immune response produced upon first exposure to a specific antigen
- **primary stain** refers, in differential staining techniques, to the first dye added to the specimen
- **primary structure** bonding sequence of amino acids in a polypeptide chain **protein** macromolecule that results when the number of amino acids linked together becomes very large, or when multiple polypeptides are used as building subunits
- **primary transcript** RNA molecule directly synthesized by RNA polymerase in eukaryotes before undergoing the additional processing required to become a mature mRNA molecule
- **primase** RNA polymerase enzyme that synthesizes the RNA primer required to initiate DNA synthesis

- **primer** short complementary sequence of five to 10 RNA nucleotides synthesized on the template strand by primase that provides a free 3'-OH group to which DNA polymerase can add DNA nucleotides
- **prion** acellular infectious particle consisting of just proteins that can cause progressive diseases in animals and humans
- **prodromal period** second stage of acute disease, during which the pathogen continues to multiply in the host and nonspecific signs and symptoms become observable
- **progeny virus** newly assembled virions ready for release outside the cell
- **proglottid** body segment of a cestode (tapeworm)
- **prokaryote** an organism whose cell structure does not include a membrane-bound nucleus
- **prokaryotic cell** a cell lacking a nucleus bound by a complex nuclear membrane
- **promoter** DNA sequence onto which the transcription machinery binds to initiate transcription
- **propagated spread** the progression of an infectious disease from person to person, either indirectly or directly, through a population of susceptible individuals as one infected individual transmits the agent to others, who transmit it to others yet again
- **prophage** phage genome that has incorporated into the host genome
- **prospective study** a research design that follows cases from the beginning of the study through time to associate measured variables with outcomes
- **prostate gland** gland that contributes fluid to semen
- **prostatitis** inflammation of the prostate gland
- **protease** enzyme involved in protein catabolism that removes individual amino acids from the ends of peptide chains
- **protease inhibitor** class of antiviral drugs, used in HIV therapy and hepatitis C therapy, that inhibits viral-specific proteases, preventing viral maturation
- **protein signature** an array of proteins expressed by a cell or tissue under a specific condition
- **Proteobacteria** phylum of gram-negative bacteria
- **proteomic analysis** study of all accumulated proteins of an organism

- **proteomics** the study of the entire complement of proteins in an organism; involves monitoring differences in gene expression patterns between cells at the protein level
- **protists** informal name for diverse group of eukaryotic organisms, including unicellular, colonial, and multicellular types that lack specialized tissues
- **proton motive force** electrochemical gradient formed by the accumulation of hydrogen ions (also known as protons) on one side of a membrane relative to the other
- **protozoan (plural: protozoa)** a unicellular eukaryotic organism, usually motile
- **protozoans** informal term for some protists, generally those that are nonphotosynthetic, unicellular, and motile protozoology the study of protozoa
- **provirus** animal virus genome that has integrated into the host chromosome
- **pseudohyphae** short chains of yeast cells stuck together
- **pseudomembrane** grayish layer of dead cells, pus, fibrin, red blood cells, and bacteria that forms on mucous membranes of the nasal cavity, tonsils, pharynx, and larynx of individuals with diphtheria
- **pseudomembranous colitis** inflammation of the large intestine with the formation of a pseudomembrane; caused by *C. difficile*
- **pseudopodia** temporary projections involved in ameboid movement; these “false feet” form by gel-sol cycling of actin polymerization/depolymerization
- **psittacosis** zoonotic *Chlamydophila* infection from birds that causes a rare form of pneumonia
- **psoriasis** autoimmune disease involving inflammatory reactions in and thickening of skin
- **psychrophile** a microorganism that grows best at cold temperatures; most have an optimum growth temperature of about 15 °C and can survive temperatures below 0 °C; most cannot survive temperatures above 20 °C
- **psychrotroph** a microorganism that grows best at cool temperatures, typically between about 4 °C and 25 °C, with optimum growth at about 20 °C

- **puerperal sepsis** sepsis associated with a bacterial infection incurred by a woman during or after childbirth
- **purines** nitrogenous bases containing a double-ring structure with a six-carbon ring fused to a five-carbon ring; includes adenine and guanine
- **purple nonsulfur bacteria** phototrophic bacteria that are similar to purple sulfur bacteria except they use hydrogen rather than hydrogen sulfide for oxidation
- **purple sulfur bacteria** phototrophic bacteria that oxidize hydrogen sulfide into elemental sulfur and sulfuric acid; their purple color is due to the pigments bacteriochlorophylls and carotenoids
- **purulent** an infection that produces pus; suppurative
- **pus** accumulation of dead pathogens, neutrophils, tissue fluid, and other bystander cells that may have been killed by phagocytes at the site of an infection
- **pyelonephritis** an infection of one or both kidneys
- **pyocyanin** blue pigments produced by some strains of *Pseudomonas aeruginosa*
- **pyoderma** any suppurative (pus-producing) infection of the skin
- **pyoverdinin** a water-soluble, yellow-green or yellow-brown pigment produced by some strains of *Pseudomonas aeruginosa*
- **pyrimidines** nitrogenous bases containing a single six-carbon ring; includes cytosine and thymine in DNA
- **pyrophosphate (PPi)** two connected phosphate groups in solution
- **pyuria** pus or white blood cells in the urine

Q

- **Q fever** highly infectious zoonotic disease caused by *Coxiella burnetii* that farmers can contract from their animals by inhalation
- **quarantine** the isolation of an individual for the purpose of preventing the spread of disease
- **quaternary ammonium salts (quats)** group of cationic detergents, named for the characteristic quaternary nitrogen atom that confers a positive charge, that make up an important class of disinfectants and antiseptics

- **quaternary structure** structure of protein complexes formed by the combination of several separate polypeptides or subunits
- **quinolines** class of antiprotozoan drugs long used for the treatment of malaria; interferes with heme detoxification
- **quorum sensing** cell-to-cell communication in bacteria; enables a coordinated response from cells when the population reaches a threshold density

R

- **R plasmid** plasmid containing genes encoding proteins that make a bacterial cell resistant to one or more antibiotics
- **rabies** contagious viral disease primarily transmitted by the bite of infected mammals that can cause acute encephalitis resulting in madness, aggressiveness, coma, and death
- **radial immunodiffusion** precipitin reaction in which antigen added to a well in an antiserum-impregnated gel diffuses, producing a precipitin ring whose diameter squared is directly proportional to antigen concentration
- **rat-bite fever** relapsing fever caused by either *Bacillus moniliformis* or *Spirillum minor*; can be transmitted by the bite of a rat or through contact with rat feces or urine
- **reaction center** protein complex in a photosystem, containing a pigment molecule that can undergo oxidation upon excitation by a light-harvesting pigment, actually giving up an electron
- **reactivation tuberculosis** secondary infection by *Mycobacterium tuberculosis* that forms later in life; occurs when the bacteria escape from the Ghon complexes and establish focal infections at other sites in immunocompromised individuals
- **reactive oxygen species (ROS)** unstable and toxic ions and molecules derived from partial reduction of oxygen
- **reading frame** way nucleotides in mRNA are grouped into codons
- **real-time PCR (quantitative PCR, qPCR)** a variant of PCR involving the use of fluorescence to allow for the monitoring of the increase in double-stranded template during a PCR reaction as it occurs, allowing for the quantitation of the original target sequence

- **receptor-mediated endocytosis** a type of endocytosis in which extracellular ligands are targeted to specific cells through their binding to specific cell surface receptors
- **recognition site** a specific, often palindromic, DNA sequence recognized by a restriction enzyme that is typically four to six base pairs long and reads the same in the 5' to 3' direction on one strand as it does in the 5' to 3' direction on the complementary strand
- **recombinant DNA molecule** a DNA molecule resulting from the cutting and insertion of DNA from one organism into the DNA of another organism, resulting in a new combination of genetic material
- **recombinant DNA pharmaceuticals** pharmaceuticals produced as a result of genetic engineering
- **recombinant DNA technology** the process by which DNA from one organism is cut and new pieces of foreign DNA from a second organism are inserted, artificially creating new combinations of genetic material within the organism
- **redox potential** tendency for a molecule to acquire electrons and become reduced; electrons flow from molecules with lower redox potentials to those with higher redox potentials
- **redox reaction** pairing of an oxidation reaction with a reduction reaction
- **reduction reaction** chemical reaction that adds electrons to acceptor molecules, leaving them reduced
- **reemerging infectious disease** a disease that was once under control or largely eradicated that has begun causing new outbreaks due to changes in susceptible populations, the environment, or the pathogen itself
- **reflection** when light bounces back from a surface
- **refraction** bending of light waves, which occurs when a light wave passes from one medium to another
- **refractive index** a measure of the magnitude of slowing of light waves by a particular medium
- **regulatory T cells** class of T cells that are activated by self-antigens and serve to inhibit peripheral self-reacting T cells from causing damage and autoimmunity
- **rejection** process by which adaptive immune responses recognize transplanted tissue as non-self, mounting a response that destroys the

tissue or leads to the death of the individual

- **relapsing fever** louse- or tickborne disease caused by *Borrelia recurrentis* or *B. hermsii* and characterized by a recurrent fever
- **replica plating** plating technique in which cells from colonies growing on a complete medium are inoculated onto various types of minimal media using a piece of sterile velvet, ensuring that the orientation of cells deposited on all plates is the same so that growth (or absence thereof) can be compared between plates
- **replication bubble** circular structure formed when the DNA strands are separated for replication
- **replication fork** Y-shaped structure that forms during the process of replication as DNA unwinds and opens up to separate the DNA strands
- **replication** process by which DNA is copied
- **reporter genes** genes that encode easily observable characteristics, allowing for their expression to be easily monitored
- **repressible operon** bacterial operon, that typically containing genes encoding enzymes required for a biosynthetic pathway and that is expressed when the product of the pathway continues to be required but is repressed when the product of the pathway accumulates, removing the need for continued expression
- **repressor** protein that suppresses transcription of a gene or operon in response to an external stimulus
- **reservoir** a living host or nonliving site in which a pathogenic organism can survive or multiply
- **resident microbiota** microorganisms that constantly live in the human body
- **resolution** the ability to distinguish between two points in an image
- **restriction endonuclease (restriction enzyme)** bacterial enzyme that cuts DNA fragments at a unique, often palindromic, recognition site; used in genetic engineering for splicing DNA fragments together into recombinant molecules
- **restriction fragment length polymorphism (RFLP)** a genetic variant identified by differing numbers or sizes of DNA fragments generated after digestion of a DNA sample with a restriction endonuclease; the variants are caused by the loss or gain of restriction sites, or the insertion or deleting of sequences between restriction sites.

- **retort** large industrial autoclave used for moist heat sterilization on a large scale
- **retrospective study** a research design that associates historical data with present cases
- **retrovirus** positive ssRNA virus that produces and uses reverse transcriptase to make an ssDNA copy of the retroviral genome that can then be made into dsDNA and integrate into the host cell chromosome to form a provirus within the host chromosome.
- **reverse transcriptase** enzyme found in retroviruses that can make a copy of ssDNA from ssRNA
- **reverse transcriptase inhibitor** classes of antiviral drugs that involve nucleoside analog competitive inhibition and non-nucleoside noncompetitive inhibition of the HIV reverse transcriptase
- **reverse transcriptase PCR (RT-PCR)** a variation of PCR used to obtain DNA copies of a specific mRNA molecule that begins with the conversion of mRNA molecules to cDNA by the enzyme reverse transcriptase
- **Reye syndrome** potentially life-threatening sequelae to some viral infections that result in the swelling of the liver and brain; aspirin use has also been linked to this syndrome
- **Rh factor** red blood cell surface antigen that can trigger type II hypersensitivity reactions
- **rheostat** a dimmer switch that controls the intensity of the illuminator on a light microscope
- **rheumatic fever** serious clinical sequela of an infection with *Streptococcus pyogenes* that can result in damage to joints or the valves of the heart
- **rheumatoid arthritis** systemic autoimmune disease in which immune complexes form and deposit in the joints and their linings, leading to inflammation and destruction
- **rhinitis** inflammation of the nasal cavity
- **rhizines** structures made of hyphae found on some lichens; aid in attachment to a surface
- **ribonucleic acid (RNA)** single-stranded nucleic acid composed of ribonucleotides; important in transcription and translation (protein synthesis)

- **ribonucleotides** RNA nucleotides containing ribose as the pentose sugar component and a nitrogenous base
- **ribosome** a complex intracellular structure that synthesizes proteins
- **riboswitch** small region of noncoding RNA found within the 5' end of some prokaryotic mRNA molecules that may bind to a small intracellular molecule, influencing the completion of transcription and/or translation
- **ribulose biphosphate carboxylase (RuBisCO)** first enzyme of the Calvin cycle responsible for adding a CO₂ molecule onto a five-carbon ribulose biphosphate (RuBP) molecule
- **rifampin** semisynthetic member of the rifamycin class that blocks bacterial RNA polymerase activity, inhibiting transcription
- **rimantadine** antiviral drug that targets the influenza virus by preventing viral escape from endosomes upon host cell uptake, preventing viral RNA release and subsequent viral replication
- **ringworm** a tinea (cutaneous mycosis of the skin), typically characterized by a round, red, slightly raised lesion that heals outward from the center, giving it the appearance of a round worm
- **RNA interference (RNAi)** process by which antisense RNAs or small interfering RNAs (siRNAs) interfere with gene expression by binding to mRNA, preventing translation and protein synthesis
- **RNA polymerase** enzyme that adds nucleotides to the 3'-OH group of the growing mRNA molecule that are complementary to the template strand, forming covalent phosphodiester bonds between the nucleotides in the RNA
- **RNA splicing** process of removing intron-encoded RNA sequences from eukaryotic primary transcripts and reconnecting those encoded by exons
- **RNA transcript** mRNA produced during transcription
- **Rocky Mountain spotted fever** potentially fatal tickborne disease caused by *Rickettsia rickettsii* characterized by fever, body aches, and a rash
- **rogue form** misfolded form of the PrP protein that is normally found in the cell membrane and has the tendency to aggregate in neurons, causing extensive cell death and brain damage
- **rolling circle replication** type of rapid unidirectional DNA synthesis of a circular DNA molecule

- **roseola** a rash-causing illness, most commonly affecting children, associated with human herpesvirus 6 (HHV-6)
- **rough endoplasmic reticulum** a type of endoplasmic reticulum containing bound 80S ribosomes for the synthesis of proteins destined for the plasma membrane
- **route of administration** method used to introduce a drug into the body
- **rRNA** type of stable RNA that is a major constituent of ribosomes, ensuring proper alignment of the mRNA and the ribosomes as well as catalyzing the formation of the peptide bonds between two aligned amino acids during protein synthesis
- **rubella** German measles, caused by the rubella virus
- **runs (running)** purposeful, directional movement of a prokaryotic cell propelled by counterclockwise flagellar rotation

S

- **σ factor** subunit of bacterial RNA polymerase conferring promoter specificity that can be substituted with a different version in response to an environmental condition, allowing for a quick and global change of the regulon transcribed
- **saccharide** carbohydrate
- **salmonellosis** gastrointestinal illness caused by *Salmonella* bacteria
- **salpingitis** inflammation of the fallopian tubes
- **sandwich ELISA** EIA in which the primary antibody is first attached to the wells of a microtiter plate, allowing it to capture antigen from an unknown solution to be quantified
- **Sanger DNA sequencing (dideoxy method, chain termination method)** the original DNA sequencing technique in which dideoxy nucleotides, each labeled with a molecular beacon, are used to terminate chain elongation; the resulting incrementally sized fragments are then separated by electrophoresis to determine the sequence of the DNA molecule
- **sanitization** protocol that reduces microbial load on inanimate surfaces to levels deemed safe for public health

- **saprozoic** refers to protozoans that ingest small, soluble food molecules
- **SARS** severe acute respiratory syndrome; caused by a zoonotic coronavirus that results in flu-like symptoms
- **saturated fatty acid** lipid with hydrocarbon chains containing only single bonds, which results in the maximum number of hydrogen atoms per chain
- **scanning electron microscope (SEM)** a type of electron microscope that bounces electrons off of the specimen, forming an image of the surface
- **scanning probe microscope** a microscope that uses a probe that travels across the surface of a specimen at a constant distance while the current, which is sensitive to the size of the gap, is measured
- **scanning tunneling microscope** a microscope that uses a probe that is passed just above the specimen as a constant voltage bias creates the potential for an electric current between the probe and the specimen
- **scarlet fever** bacterial infection caused by *Streptococcus pyogenes*, marked by a high fever and a disseminated scarlet rash
- **schistosomiasis** helminthic infection caused by *Schistosoma* spp.; transmitted from a snail intermediate host to human swimmers or bathers in freshwater
- **schizogony** asexual reproduction in protozoans that is characterized by multiple cell divisions (one cell dividing to form many smaller cells)
- **scolex** the head region of a cestode (tapeworm), which typically has suckers and/or hooks for attachment to the host
- **scrapie** form of transmissible spongiform encephalopathy that primarily affects sheep
- **sebaceous gland** a gland located in hair follicles that secretes sebum
- **sebum** lipid-rich substance secreted by the sebaceous glands of the skin
- **secondary antibody** antibody to which an enzyme is attached for use in ELISA assays; in direct and sandwich ELISAs, it is specific for the antigen being quantified, whereas in indirect ELISA, it is specific for the primary antibody
- **secondary immunodeficiency** impaired immune response due to infection, metabolic disturbance, poor diet, stress, or other acquired factors

- **secondary infection** second infection that develops after a primary infection as a result of the primary disease compromising immune defenses or antibiotics, thus eliminating protective microbiota
- **secondary lymphoid tissue** one of two types of lymphatic tissue; comprises the spleen, lymph nodes, Peyer's patches, and mucosa associated lymphoid tissue (MALT)
- **secondary response** the adaptive immune response produced in response to a specific antigen to which the body has previously been exposed
- **secondary structure** structure stabilized by hydrogen bonds between the carbonyl and amine groups of a polypeptide chain; may be an α -helix or a β -pleated sheet, or both
- **secretory vesicle** membranous sac that carries molecules through the plasma membrane to be released (secreted) from the cell
- **selective IgA deficiency** primary immunodeficiency in which individuals produce normal levels of IgG and IgM, but are unable to produce secretory IgA
- **selective media** media that contain additives that encourage the growth of some bacteria while inhibiting others
- **selective toxicity** desirable quality of an antimicrobial drug indicating that it preferentially kills or inhibits the growth of the target microbe while causing minimal or no harm to the host
- **semiconservative DNA replication** pattern of DNA replication process whereby each of the two parental DNA strands acts as a template for new DNA to be synthesized, producing hybrid old- and new-strand daughter molecules
- **semicritical item** object that contacts mucous membranes or nonintact skin but does not penetrate tissues; requires a high level of disinfection
- **seminal vesicles** glands that contribute fluid to semen
- **semisynthetic antimicrobial** chemically modified derivative of a natural antibiotic
- **sense strand** strand of DNA that is not transcribed for gene expression; it is complementary to the antisense strand
- **sepsis** systemic inflammatory response to an infection that results in high fever and edema, causing organ damage and possibly leading to shock and death

- **septate hyphae** hyphae that contain walls between individual cells; characteristic of some fungi
- **septic arthritis** see *infectious arthritis*
- **septic shock** serious condition marked by the loss of blood pressure resulting from an inflammatory response against a systemic infection
- **septic** the condition of being septicemic; having an infection in the blood
- **septicemia** condition in which pathogens are multiplying in blood
- **septicemic plague** form of plague that occurs when the bacterial pathogen gains access to the bloodstream
- **septum** separating structure that forms during cell division; also describes the separating wall between cells in a filament
- **sequela (plural: sequelae)** condition that arises as a consequence of a prior disease
- **serial dilution** sequential transfer of known volumes of culture samples from one tube to another to perform a several-fold dilution of the original culture
- **seroconversion** point in an infection at which antibody to a pathogen is detectible using an immunoassay
- **serotype** strain or variation of the same species of bacteria; also called serovar
- **serovar** specific strain of bacteria identified by agglutination using strain-specific antisera
- **serum** fluid portion of the blood after clotting has occurred; generally lacks clotting factors
- **serum sickness** systemic type III hypersensitivity reaction
- **sessile** attached to a surface
- **severe combined immunodeficiency disease (SCID)** genetic disorder resulting in impaired function of B cells and T cells
- **sex pilus** specialized type of pilus that aids in DNA transfer between some prokaryotic cells
- **sheath** part of the tail on a bacteriophage that contracts to introduce the viral DNA into the bacterium
- **shigellosis** gastrointestinal illness caused by *Shigella* bacteria, also called bacillary dysentery
- **shingles** acute and painful rash that forms following the reactivation of a latent chickenpox infection

- **shock** extreme drop in blood pressure that, among other causes, can result from a strong immune response to the activity of toxins or response to bacterial products and can result in death
- **shuttle vector** a plasmid that can move between bacterial and eukaryotic cells
- **side chain** the variable functional group, R , attached to the α carbon of an amino acid
- **sign** objective and measurable indication of a disease
- **silent mutation** point mutation that results in the same amino acid being incorporated into the resulting polypeptide
- **simple microscope** a type of microscope with only one lens to focus light from the specimen
- **simple staining** a staining technique that uses a single dye
- **single-stranded binding protein** protein that coats the single strands of DNA near each replication fork to prevent the single-stranded DNA from rewinding into a double helix
- **sinusitis** inflammation of the sinuses
- **S-layer** cell envelope layer composed of protein covering the cell walls of some bacteria and archaea; in some archaea, may function as the cell wall
- **slime layer** a type of glycocalyx with unorganized layers of polysaccharides that aid bacterial adherence to surfaces
- **smear** a thin layer of a specimen on a slide
- **smooth endoplasmic reticulum** a type of endoplasmic reticulum that lacks ribosomes, is involved in the biosynthesis of lipids and in carbohydrate metabolism, and serves as the site of detoxification of toxic compounds within the cell
- **soft chancres** soft, painful ulcers associated with the STI chancroid
- **soma** cell body of a neuron
- **sonication** method of microbial control that involves application of ultrasound waves to form cavitation within a solution, including inside cells, disrupting cell components as a result
- **Southern blot** a technique in molecular genetics used to detect the presence of certain DNA sequences within a given DNA sample; DNA fragments within the sample are separated by agarose gel electrophoresis, immobilized on a membrane, and then exposed to a

specific DNA probe labeled with a radioactive or fluorescent molecular beacon to aid in detection

- **specialized transduction** transfer of a specific piece of bacterial chromosomal DNA near the site of integration by the phage
- **specificity** the ability of the specific adaptive immune system to target specific pathogens or toxins
- **spike** viral glycoprotein embedded within the viral capsid or envelope used for attachment to host cells
- **spirochetes** a group of long, thin, spiral-shaped fastidious bacteria that includes the human pathogens that cause syphilis, Lyme disease, and leptospirosis
- **spleen** abdominal organ consisting of secondary lymphoid tissue that filters blood and captures pathogens and antigens that pass into it; also contains specialized macrophages and dendritic cells that are crucial for antigen presentation
- **spliceosome** protein complex containing small nuclear ribonucleoproteins that catalyzes the splicing out of intron-encoded RNA sequences from the primary transcript during RNA maturation in eukaryotes
- **spontaneous generation** the now-disproven theory that life can arise from nonliving matter
- **spontaneous mutation** mutation not caused by a mutagen that occurs through DNA replication errors
- **sporadic disease** an illness that occurs at relatively low levels with no discernible pattern or trend, frequently with no geographic focus
- **spores** specialized cells that may be used for reproduction or may be specialized to withstand harsh conditions
- **sporotrichosis** subcutaneous infection caused by the fungus *Sporothrix schenckii*, which causes skin lesions and can potentially spread to the lymphatic system; also known as rose gardener's disease or rose thorn disease
- **sporulation** the process by which a vegetative cell produces a dormant endospore
- **spread plate method** a technique used for inoculating plates with diluted bacterial samples for the purpose of cell counting; the liquid sample is pipetted onto solid medium and spread uniformly across the plate

- **St. Louis encephalitis** mosquito-borne viral infection of the brain that occurs primarily in the central and southern United States
- **stage** the platform of a microscope on which slides are placed
- **staining** the addition of stains or dyes to a microscopic specimen for the purpose of enhancing contrast
- **staphylococcal food poisoning** gastrointestinal illness caused by toxins produced by *Staphylococcus aureus*
- **staphylolysins** a class of staphylococcal exotoxins that are cytotoxic to skin cells and white blood cells
- **starch** energy-storage polysaccharide in plants; composed of two types of glucose polymers: amylose and amylopectin
- **start codon** AUG codon, specifying methionine, which is typically the codon that initiates translation
- **stationary phase** interval during which the number of cells formed by cell division is equal to the number of cells dying
- **stereoisomers** isomers that differ in the spatial arrangements of atoms
- **sterilant** strong chemical that effectively kills all microbes and viruses in or on an inanimate item
- **sterile field** specified area that is free of all vegetative microbes, endospores, and viruses
- **sterilization** protocol that completely removes all vegetative cells, endospores, and viruses from an item
- **steroid** lipid with complex, ringed structures found in cell membranes and hormones
- **sterol** the most common type of steroid; contains an OH group at one specific position on one of the molecule's carbon rings
- **sticky ends** short, single-stranded complementary overhangs that may be produced when many restriction enzymes cut DNA
- **stigma** light-sensing eyespot found in *Euglena*
- **stop codon (nonsense codon)** one of three codons for which there is no tRNA with a complementary anticodon; a signal within the mRNA for termination of translation
- **stratum corneum** a layer of dead, keratinized cells that forms the uppermost layer of the epidermis
- **strep throat (streptococcal pharyngitis)** bacterial pharyngitis caused by *Streptococcus pyogenes*

- **streptococcal toxic shock-like syndrome (STSS)** condition similar to staphylococcal toxic shock syndrome but with greater likelihood of bacteremia, necrotizing fasciitis, and acute respiratory distress syndrome
- **stroma** a gel-like fluid that makes up much of a chloroplast's volume, and in which the thylakoids floats
- **strongyloidiasis** soil-transmitted intestinal infection caused by the helminth *Strongyloides stercoralis*
- **structural formula** graphic representation of the molecular structure showing how the atoms are arranged
- **structural isomers** molecules composed of the same numbers and types of atoms but with different bonding sequences
- **subacute bacterial endocarditis** form of endocarditis in which damage to the valves of the heart occurs over months as a result of blood clot formation and immune-response-induced fibrosis of the valves
- **subclinical disease** disease that does not present any signs or symptoms
- **subcutaneous mycosis** any fungal infection that penetrates the epidermis and dermis to enter deeper tissues
- **substrate** chemical reactants of an enzymatic reaction
- **substrate-level phosphorylation** direct method of ATP production in which a high-energy phosphate group is removed from an organic molecule and added to an ADP molecule
- **subunit vaccine** vaccine that contains only key antigens as opposed to whole pathogens
- **sugar-phosphate backbone** alternating sugar-phosphate structure composing the framework of a nucleic acid strand that results from phosphodiester bond formation between nucleotides
- **sulfonamides (sulfa drugs)** group of structurally related synthetic antimicrobial compounds that function as antimetabolites, competitively inhibiting an enzyme in the bacterial folic acid synthesis pathway
- **superantigen** class of exotoxin that triggers a strong nonspecific immune response with excessive production of cytokines (cytokine storm) causing inflammation, high fever, shock, and, potentially, death

- **supercoiled** extensive wrapping and twisting of a DNA molecule, allowing the DNA to fit within a small space
- **supercoiling** process in which DNA is underwound or overwound to fit inside a cell
- **supercritical fluid** molecule, commonly carbon dioxide, brought to high pressures to reach a state that has physical properties between those of liquids and gases, allowing it to effectively penetrate surfaces and cells to form carbonic acid, which lowers the pH of cells considerably, leading to sterilization
- **superinfection** secondary infection that may develop as a result of long-term, broad-spectrum antimicrobial use
- **superoxide dismutase** enzyme that catalyzes the breakdown of superoxide anions
- **suppurative** producing pus; purulent
- **surfactant** group of chemical compounds used for degerming; lower the surface tension of water, creating emulsions that mechanically carry away microorganisms
- **sweat gland** one of numerous tubular glands embedded in the dermis that secretes the watery substance known as perspiration
- **symbiosis** any interaction between different species that are associated with each other within a community
- **symptom** subjective experience of disease felt by the patient
- **synapse** junction between a neuron and another cell
- **syncytia** multinucleated cells that form from the fusion of normal cells during infections or other processes
- **syndrome** group of signs and symptoms characteristic of a particular disease
- **syngamy** process in which haploid gametes fuse
- **synthetic antimicrobial** antimicrobial developed from a chemical not found in nature
- **syphilis** an STI caused by the bacterium *Treponema pallidum*
- **systemic autoimmune disease** autoimmune disease that affect the organism as a whole, rather than a single organ
- **systemic infection** infection that has spread to multiple locations or body systems
- **systemic inflammatory response syndrome (SIRS)** severe inflammatory response to the presence of microbes in the blood; can

lead to sepsis

- **systemic lupus erythematosus (SLE)** systemic autoimmune disease producing inflammatory type III hypersensitivities as antibodies form immune complexes with nuclear and cytoplasmic antigens
- **systemic mycosis** a fungal infection that spreads throughout the body

T

- **T-cell receptors (TCR)** molecules on T cells involved in the recognition of processed foreign epitopes presented with MHC I or MHC II
- **T lymphocyte** lymphocyte that serves as the central orchestrator, bridging humoral, cellular, and innate immunity, and serves as the effector cells of cellular immunity; T cell
- **taeniasis** infection caused by *Taenia* or *Diphyllobothrium*
- **tail fiber** long protein component on the lower part of a phage used for specific attachment to bacterial cell
- **tail pins** points extended at the base of a bacteriophage sheath that, along with tail fibers, lead to phage attachment to a bacterial cell
- **tapeworms** segmented, hermaphroditic, parasitic flatworms (Platyhelminthes)
- **tartar** calcified heavy plaque on teeth, also called dental calculus
- **taxonomy** the classification, description, identification, and naming of living organisms
- **T-dependent antigen** a protein antigen that is only capable of activating a B cell with the cooperation of a helper T cell
- **TDP** thermal death point is the lowest temperature at which all microorganisms are killed in a 10-minute exposure
- **TDT** thermal death time is the length of time needed to kill all microorganisms in a sample at a given temperature
- **telomerase** enzyme that attaches to the end of a linear chromosome and adds nucleotides to the 3' end of one of the DNA strands, maintaining the telomere sequence, thus preventing loss of DNA from the end of the chromosome
- **telomere** repetitive, noncoding sequence found at the end of a linear eukaryotic chromosome that protects the genes near the end of the

chromosome from deletion as the DNA molecule is repeatedly replicated

- **temperate phage** bacteriophage that can incorporate viral genome into the host cell chromosome and replicate with the host cell until new viruses are produced; a phage that undergoes the lysogenic cycle
- **teratogenic** able to disrupt the normal development of a fetus in utero
- **terbinafine** antifungal drug of the allylamine class that is used topically for the treatment of dermatophytic skin infections
- **termination of DNA replication** stage of replication during which DNA replication is halted once the chromosome has been fully replicated
- **termination of transcription** stage of transcription that occurs when RNA polymerase has reached specific DNA sequences, leading to release of the enzyme from the DNA template, freeing the RNA transcript and, thus, halting transcription
- **termination of translation** stage of translation during which a nonsense codon aligns with the A site, signaling release factors to release of the polypeptide, leading to the dissociation of the small and large ribosomal subunits from the mRNA and from each other
- **tertiary structure** large-scale, three-dimensional structure of a polypeptide
- **test sensitivity** probability that a diagnostic test will find evidence of the targeted disease when the pathogen is present
- **test specificity** probability that a diagnostic test will not find evidence of the targeted disease when the pathogen is absent
- **testes** (singular *testis*) pair of glands located in the scrotum of males that produce sperm and testosterone
- **tetanus** bacterial disease caused by exotoxin produced by *Clostridium tetani* that causes a rigid paralysis
- **tetracyclines** class of protein synthesis inhibitors that bind to the 30S subunit, blocking the association of tRNAs with the ribosome during translation
- **T_H1 cells** subtype of T cells that stimulate cytotoxic T cells, macrophages, neutrophils, and NK cells
- **T_H17 cells** subtype of T cell that are essential for defense against specific pathogens and infections, such as chronic mucocutaneous infections with *C. albicans*

- **T_H2 cells** subtype of T cells that stimulate B cells and direct their differentiation; also involved in directing antibody class switching
- **thallus** body of fleshy fungi (more generally, a body without a root, stem, or leaf) that commonly co-occurs with HIV infection; the microbes move to the lymphatic system in the groin
- **thermophile** a microorganism that grows best at warm temperatures, typically between about 50 °C and 80 °C
- **thin sections** thin slices of tissue for examination under a TEM
- **thioglycolate medium** medium designed to test the aerotolerance of bacteria; it contains a low concentration of agar to allow motile bacteria to move throughout the medium
- **thioglycolate tube culture** contains reducing medium through which oxygen diffuses from the tube opening, producing a range of oxygen environments down the length of the tube
- **thrombocytes** see *platelets*
- **thylakoids** a highly dynamic collection of membranous sacs found in the stroma of chloroplasts; site of photosynthesis
- **thymic selection** a three-step process of negative and positive selection of T cells in the thymus
- **thymine dimer** covalent linkage between two adjacent thymine bases on exposure to ultraviolet radiation
- **thymine** pyrimidine nitrogenous base found only in DNA nucleotides
- **tincture** solution of an antiseptic compound dissolved in alcohol
- **T-independent antigen** a nonprotein antigen that can activate a B cell without cooperation from a helper T cell
- **tinea** any cutaneous fungal infection caused by dermatophytes, such as tinea corporis, tinea capitis, tinea cruris, and tinea pedis
- **tinea capitis** cutaneous mycosis of the scalp; also known as ringworm of the scalp
- **tinea corporis** cutaneous mycosis of the body; also known as ringworm of the body
- **tinea cruris** cutaneous mycosis of the groin region; also known as jock itch
- **tinea pedis** cutaneous mycosis of the feet; also known as athlete's foot
- **tissue tropism** tendency of most viruses to infect only certain tissue types within a host

- **titer** concentration obtained by titration; the reciprocal of a measurement of biological activity determined by finding the dilution of an unknown (e.g., antigen-specific antibody in an antiserum) that shows the defined end-point; always expressed as a whole number
- **tolerance** lack of an anti-self immune response
- **toll-like receptors (TLRs)** pathogen recognition receptors (PRRs) that may be found on the external surface of phagocytes or facing inward in interior compartments
- **tonsillitis** inflammation of the tonsils
- **topoisomerase** type of enzyme that helps maintain the structure of supercoiled chromosomes, preventing overwinding of DNA during certain cellular processes like DNA replication
- **topoisomerase II** enzyme responsible for facilitating topological transitions of DNA, relaxing it from its supercoiled state
- **total magnification** in a light microscope is a value calculated by multiplying the magnification of the ocular by the magnification of the objective lenses
- **toxemia** presence of toxins in the blood
- **toxic shock syndrome** severe condition marked by the loss of blood pressure and blood clot formation caused by a bacterial superantigen, toxic shock syndrome toxin
- **toxigenicity** ability of a pathogen to produce toxins to cause damage to host cells
- **toxin** poison produced by a pathogen
- **toxoid vaccine** vaccine that contains inactivated bacterial toxins
- **toxoplasmosis** typically asymptomatic protozoan infection caused by *Toxoplasma* spp. and transmitted through contact with cysts in cat feces; infections in pregnant women may cause birth defects or miscarriage
- **trace element** indispensable element present in cells in lower amounts than macronutrients; also called *micronutrient*
- **trachea** also known as the windpipe, this is a stiffened tube of cartilage that runs from the larynx to the bronchi
- **trachoma** a type of conjunctivitis, caused by *Chlamydia trachomatis*, that is a major cause of preventable blindness
- **transcription bubble** region of unwinding of the DNA double helix during transcription

- **transcription factors** proteins encoded by regulatory genes that function by influencing the binding of RNA polymerase to the promoter and allowing its progression to transcribe structural genes
- **transcription** process of synthesizing RNA using the information encoded in DNA
- **transcriptomics** the study of the entire collection of mRNA molecules produced by cells; involves monitoring differences in gene expression patterns between cells at the mRNA level
- **transduction** mechanism of horizontal gene transfer in bacteria in which genes are transferred through viral infection
- **transendothelial migration** process by which circulating leukocytes exit the bloodstream via the microvascular endothelium
- **transfection** the introduction of recombinant DNA molecules into eukaryotic hosts
- **transformation** mechanism of horizontal gene transfer in bacteria in which naked environmental DNA is taken up by a bacterial cell
- **transgenic** describing an organism into which foreign DNA from a different species has been introduced
- **transient microbiota** microorganisms, sometimes pathogenic, that are only temporarily found in the human body
- **transition reaction** reaction linking glycolysis to the Krebs cycle, during which each pyruvate is decarboxylated and oxidized (forming NADH), and the resulting two-carbon acetyl group is attached to a large carrier molecule called coenzyme A, resulting in the formation of acetyl-CoA and CO₂; also called the *bridge reaction*
- **translation (protein synthesis)** process of protein synthesis whereby a ribosome decodes an mRNA message into a polypeptide product
- **transmissible spongiform encephalopathy** degenerative disease caused by prions; leads to the death of neurons in the brain
- **transmission electron microscope (TEM)** a type of electron microscope that uses an electron beam, focused with magnets, that passes through a thin specimen
- **transmittance** the amount of light that passes through a medium
- **transparency** the property of allowing light to pass through
- **transport vesicle** membranous sac that carries molecules between various components of the endomembrane system

- **transposition** process whereby a DNA sequence known as a transposon independently excises from one location in a DNA molecule and integrates elsewhere
- **transposon (transposable element)** molecule of DNA that can independently excise from one location in a DNA molecule and integrate into the DNA elsewhere
- **trench fever** louseborne disease caused by *Bartonella quintana* and characterized by high fever, body aches, conjunctivitis, ocular pain, severe headaches, and severe bone pain
- **trench mouth** a severe form of gingivitis, also called acute necrotizing ulcerative gingivitis
- **treponemal serologic tests** tests for syphilis that measure the amount of antibody directed against antigens associated with *Treponema pallidum*
- **triacylglycerol** three fatty acids chemically linked to a glycerol molecule; also called a triglyceride
- **triazoles** ergosterol biosynthesis inhibitors used to treat several types of systemic yeast infections; exhibit more selective toxicity than the imidazoles and are associated with fewer side effects
- **tricarboxylic acid cycle** see *Krebs cycle*
- **trichinosis** soil-transmitted intestinal infection caused by the nematode *Trichinella spiralis*; associated with cyst formation
- **trichomoniasis** a common STI caused by *Trichomonas vaginalis*
- **trichuriasis** intestinal infection caused by the whipworm *Trichuris trichiura*
- **triglyceride** three fatty acids chemically linked to a glycerol molecule; also called a triacylglycerol
- **trimethoprim** synthetic antimicrobial compound that functions as an antimetabolite to an enzyme in the bacterial folic acid synthesis pathway
- **tRNA** small type of stable RNA that carries the correct amino acid to the site of protein synthesis in the ribosome and base pairs with the mRNA to allow the amino acid it carries to be inserted in the polypeptide chain being synthesized
- **trophozoite** a life cycle phase in which protists are actively feeding and growing
- **tubercle** small, rounded lesion

- **tuberculosis** life-threatening form of microbial infection marked by the presence of acid-fast bacteria growing in nodules (especially in the lungs)
- **tularemia** infection of the lymphatic system by *Francisella tularensis*; also known as rabbit fever
- **tumbles (tumbling)** random, circuitous movement of a bacterial cell, propelled by clockwise flagellar rotation
- **tumor** collection or aggregate of cells; can be benign (noncancerous) or malignant (cancerous)
- **tumor-inducing (Ti) plasmid** a naturally occurring plasmid of the bacterium *Agrobacterium tumefaciens* that researchers use as a shuttle vector to introduce a desired DNA fragment into plant cells
- **turbidity** cloudiness of a culture due to refraction of light by cells and particles
- **two-photon microscope** a microscope that uses long-wavelength or infrared light to fluoresce fluorochromes in the specimen
- **tympenic membrane** also referred to as the ear drum, this structure separates the outer and middle ear
- **type 1 diabetes mellitus** hyperglycemia caused by an autoimmune disease affecting insulin production by β cells of the pancreas
- **type I hypersensitivity** rapid-onset allergic reaction due to cross-linking of antigen-specific IgE on the outside of mast cells, resulting in release of inflammatory mediators
- **type II hypersensitivity** cytotoxic reaction triggered by IgG and IgM antibodies binding to antigens on cell surfaces
- **type III hypersensitivity** inflammatory reaction induced by formation of immune complexes and their deposition in tissues and blood vessels
- **type IV hypersensitivity** delayed T-cell-mediated inflammatory reaction that takes longer to manifest than the first three hypersensitivity types, due to the need for activation of antigen-presenting cell and T-cell subsets
- **typhoid fever** serious illness caused by infection with certain serotypes of *Salmonella*

U

- **UHT pasteurization** method of pasteurization that exposes milk to ultra-high temperatures (near 140 °C) for a few seconds, effectively sterilizing it so that it can be sealed and stored for long periods without refrigeration
- **ulcer** open sore
- **ultramicrotome** a device that cuts thin sections for electron microscopy
- **unit membrane** biological membrane composed of two layers of phospholipid molecules with the nonpolar tails associating to form a hydrophobic barrier between the polar heads; also called lipid bilayer
- **unsaturated fatty acid** lipid with hydrocarbon chains containing one or more carbon-carbon double bonds and subsequently fewer than the maximum number of hydrogen atoms per chain
- **uracil** pyrimidine nitrogenous base found only in RNA nucleotides
- **ureter** duct that transports urine from the kidneys to the urinary bladder
- **ureteritis** inflammation of the ureter
- **urethra** duct through which urine passes from the urinary bladder to leave the body through the urinary meatus
- **urethritis** inflammation of the urethra
- **urinary bladder** an organ that stores urine until it is ready to be excreted
- **urinary meatus** the opening through which urine leaves the body
- **use-dilution test** a technique for determining the effectiveness of a chemical disinfectant on a surface; involves dipping a surface in a culture of the targeted microorganism, disinfecting the surface, and then transferring the surface to a fresh medium to see if bacteria will grow
- **uterus** female reproductive organ in which a fertilized egg implants and develops

V

- **vaccination** inoculation of a patient with attenuated pathogens or antigens to activate adaptive immunity and protect against infection

- **vagina** female reproductive organ that extends from the vulva to the cervix
- **vaginitis** inflammation of the vagina
- **vaginosis** an infection of the vagina caused by overgrowth of resident bacteria
- **vancomycin** cell wall synthesis inhibitor of the glycopeptide class
- **vancomycin-intermediate *Staphylococcus aureus* (VISA)** pathogen with intermediate vancomycin resistance due to increased targets for and trapping of vancomycin in the outer cell wall
- **vancomycin-resistant enterococci (VRE)** pathogens resistant to vancomycin through a target modification of peptidoglycan subunit peptides that inhibit binding by vancomycin
- **vancomycin-resistant *Staphylococcus aureus* (VRSA)** pathogen with resistance to vancomycin that has arisen as a result of the horizontal gene transfer of vancomycin resistance genes from VRE
- **variolation** the historical practice of inoculating a healthy patient with infectious material from a person infected with smallpox in order to promote immunity to the disease
- **vas deferens** pair of ducts in the male reproductive system that conduct sperm from the testes and seminal fluid to the ejaculatory duct
- **vasculitis** inflammation affecting blood vessels (either arteries or veins)
- **VDRL (Venereal Disease Research Laboratory) test** test for syphilis that detects anti-treponemal antibodies to the phospholipids produced due to the tissue destruction by *Treponema pallidum*; antibodies are detected through a flocculation reaction with cardiolipin extracted from beef heart tissue
- **vector** animal (typically an arthropod) that transmits a pathogen from one host to another host; DNA molecules that carry DNA fragments from one organism to another
- **vegetative cell** a cell that is actively growing and dividing, and does not contain an endospore
- **vehicle transmission** transfer of a pathogen between hosts via contaminated food, water, or air
- **vein** blood vessel that returns blood from the tissues to the heart for recirculation

- **vertical direct transmission** transfer of a pathogen from mother to child during pregnancy, birth, or breastfeeding
- **vertical gene transfer** transfer of genes from parent to offspring
- **viable cell** live cell; live cells are usually detected as colony-forming units
- **viable plate count** direct method of measuring microbial growth in a culture; the number of viable or live cells is usually expressed in CFU/mL
- **viral conjunctivitis** inflammation of the conjunctiva caused by a viral infection
- **viral envelope** lipid membrane obtained from phospholipid membranes of the cell that surrounds the capsid
- **viral hemagglutination inhibition assay** assay used to quantify the amount of neutralizing antibody against a virus by showing a decrease in hemagglutination caused by a standardized amount of virus
- **viral titer** number of virions per unit volume
- **viremia** presence of virus in blood
- **viricide** chemical or physical treatment that destroys or inactivates viruses
- **virion** inert particle that is the reproductive form of a virus
- **viroid** infectious plant pathogen composed of RNA
- **virology** the study of viruses
- **virulence** degree to which an organism is pathogenic; severity of disease signs and symptoms
- **virulence factor** product of a pathogen that assists in its ability to cause infection and disease
- **virulent phage** bacteriophage for which infection leads to the death of the host cell; a phage that undergoes the lytic cycle
- **virus** an acellular microorganism, consisting of proteins and genetic material (DNA or RNA), that can replicate itself by infecting a host cell
- **virusoid** small piece of RNA associated with larger RNA of some infectious plant viruses
- **volutin** inclusions of polymerized inorganic phosphate; also called metachromatic granules
- **vulva** the female external genitalia

W

- **water activity** water content of foods or other materials
- **wavelength** the distance between one peak of a wave and the next peak
- **Weil's disease** advanced stage of leptospirosis in which the kidney and liver become seriously infected
- **West African trypanosomiasis** chronic form of African trypanosomiasis caused by *Trypanosoma brucei gambiense*
- **West Nile encephalitis** mosquito-borne disease caused by the West Nile virus (WNV) that can result in swelling of the brain and death in severe cases
- **western blot** technique used to detect the presence of a certain protein within a given protein sample in which proteins within the sample are separated by PAGE, immobilized on a membrane, and then exposed first to an antibody that binds to the protein of interest and then second to an antibody equipped with a molecular beacon that will bind to the first antibody
- **western equine encephalitis** serious but rare mosquito-borne viral infection of the brain that is found primarily in the central and western United States
- **wet mount** a slide preparation technique in which a specimen is placed on the slide in a drop of liquid
- **wheal-flare reaction** localized type I hypersensitivity reaction, involving a raised, itchy bump (wheal) and redness (flare), to injected allergen
- **whooping cough** common name for pertussis
- **wild type** phenotype of an organism that is most commonly observed in nature
- **Winterbottom's sign** acute swelling of lymph nodes at the back of the neck that is an early sign of African trypanosomiasis
- **wobble position** third position of a codon that, when changed, typically results in the incorporation of the same amino acid because of the degeneracy of the genetic code
- **World Health Organization (WHO)** international public health organization within the United Nations; monitors and communicates

international public health information and coordinates international public health programs and emergency interventions

X

- **xenobiotic** compound synthesized by humans and introduced to an environment in much higher concentrations than expected in nature
- **xenograft** transplanted tissue from a donor that is of a different species than the recipient
- **X-linked agammaglobulinemia** genetic disorder resulting in an inability to produce antibodies
- **x-y mechanical stage knobs** knobs on a microscope that are used to adjust the position of the specimen on the stage surface, generally to center it directly above the light

Y

- **yeast** any unicellular fungus
- **yeast infection** fungal infection of the vagina typically caused by an overgrowth of resident *Candida* spp.
- **yellow fever** mild to potentially fatal mosquito-borne viral disease caused by the yellow fever virus

Z

- **Ziehl-Neelsen technique** a method of acid-fast staining that uses heat to infuse the primary stain, carbolfuchsin, into acid-fast cells
- **zone of inhibition** clear zone around a filter disk impregnated with an antimicrobial drug, indicating growth inhibition due to the antimicrobial drug
- **zoonosis** see *zoonotic disease*
- **zoonotic disease** any disease that is transmitted to humans by animals
- **zooplankton** heterotrophic plankton

- **Z-scheme** electron flow seen in noncyclic photophosphorylation in plants, algae, and cyanobacteria due to the use of both PSI and PSII
- **zygospores** spores used by Zygomycetes for sexual reproduction; they have hard walls formed from the fusion of reproductive cells from two individuals